02-28-01

JC09 Rec'd PCT/PTO 27 FEB 2001 PCT

FORM PTO-1390 (MODIFIED) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER: X-12239

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5): 09/763994

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/19436	08/30/1999 (08.30.99)	08/01/1998 (08.01.98)

TITLE OF INVENTION: HUMAN LATENT TRANSFORMING GROWTH FACTOR-β BINDING PROTEIN 3

APPLICANT(S) FOR DO/EO/US: Brian Taylor Edmonds

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [X] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A copy of the International Preliminary Examination Report (IPER), including any annexes, and, if not in English, an English language translation of the annexes to the IPER under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/763994	PCT/US99/19436	X-12239

17. [X] The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **$710.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**

International preliminary examination fee paid to USPTO (37 CRF 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 690.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than __20 __30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	16 - 20=	0	**X $18.00**	$	
Independent claims	3 - 3=	0	**X $80.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			**+ $270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 690.00	
Processing fee of **$130.00** for furnishing English translation later than __ 20 __30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 690.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 690.00	
				Amount to be refunded	$
				charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.
b. [X] Please charge my Deposit Account No. 05-0840 in the amount of $ 690.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 05-0840. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
ELI LILLY AND COMPANY
PATENT DIVISION/RS
LILLY CORPORATE CENTER

SIGNATURE

Robert L. Sharp
NAME

Date

45,609
REGISTRATION NUMBER

(317) 276-5332
TELEPHONE NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Brian Taylor Edmonds
International Application No.: PCT/US99/19436
Filed: August 30, 1999 (08/30/99)
Invention: HUMAN LATENT TRANSFORMING GROWTH FACTOR-β BINDING PROTEIN 3
Lilly Reference: X-12239
Earliest Priority Date: September 1, 1998 (09/01/98)

Certificate Under 37 C.F.R. § 1.10

Attention: DO/EO
Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir/Madam:

"Express Mail" mailing label number: EL559725944US
Date of Deposit: February 27, 2001

I hereby certify that the following attached paper or fee

Transmittal Letter to the United States Designated/Elected Office (US) concerning a filing under 35 U.S.C. 371 of the International Application identified above is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Olga M. Franz
(Typed or printed name of person mailing paper)

Olga M. Franz
(Signature of person mailing paper or fee)

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date appearing below.
ELI LILLY AND COMPANY

By KS Rhoades Date 10-10-01

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Brian Taylor Edmonds	)
Serial No.	:	09/763,994	)
Filed	:	August 30, 1999	)
For	:	HUMAN LATENT TRANSFORMING GROWTH FACTOR-BETA BINDING PROTEIN 3	) Examiner: John L. Anderson
Docket No.	:	X-12239	)

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 C.F.R. 1.821(f) (SEQUENCE LISTING)

Assistant Commissioner for Patents
Washington, D. C. 20231
Sir:

I hereby affirm that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. 1.821(c) and (e), respectively, are the same.

Respectfully submitted,

Robert L. Sharp
Attorney/Agent for Applicant
Registration No. 45,609
Phone: 317-276-5332

Eli Lilly and Company
Patent Division/RLS
Lilly Corporate Center
Indianapolis, Indiana 46285

October 9, 2001

"Express Mail" mailing label number EL559725944US

Date of Deposit February 27, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Olga M Franz
Printed Name

Olga M Franz
Signature

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

The Accompanying Application

Applicant : Brian Taylor Edmonds

For : HUMAN LATENT TRANSFORMING GROWTH FACTOR-β BINDING PROTEIN 3

Docket No. : X-12239

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D. C. 20231

Sir:

Please amend the accompanying application as follows:

In the Claims

Please cancel previous claims 1-31 and add new claims 32 - 45 as set forth below:

32. An hLTBP-3 polypeptide comprising at least 181 contiguous amino acids of a polypeptide selected from the group consisting of:

a) SEQ ID NO: 2;

b) SEQ ID NO: 4;

c) SEQ ID NO: 6; and

d) a pharmaceutically acceptable salt of a), b), or c).

33. A nucleic acid encoding the hLTBP-3 polypeptide of claim 32.

34. A vector comprising the nucleic acid of claim 33.

35. A host cell comprising the vector of claim 34.

36. A method for producing a hLTBP-3 polypeptide according to claim 32 wherein said method comprises culturing at least one recombinant host cell comprising a vector comprising a nucleic acid encoding said hLTBP-3 polypeptide under conditions suitable for expression of said hLTBP-3 polypeptide.

37. An antibody that binds at least one epitope of a polypeptide selected from the group consisting of:

a) SEQ ID NO:2;
b) SEQ ID NO:4;
c) SEQ ID NO:5; and
d) SEQ ID NO:6.

38. A composition comprising at least one carrier, excipient, or diluent and an hLTBP-3 polypeptide according to claim 32.

39. A composition comprising at least one carrier, excipient, or diluent and an antibody according to claim 37.

40. A method for inhibiting tissue growth which comprises administering to a patient in need thereof a tissue growth inhibiting amount of at least one composition according to claim 38.

41. A method for inhibiting tissue growth which comprises administering to a patient in need thereof a tissue growth inhibiting amount of at least one composition according to claim 39.

42. A method for inhibiting tumor growth, which comprises administering to a patient in need thereof a tumor cell growth inhibiting effective amount of at least one composition according to claim 39.

43. A method for stimulating tissue growth *in vitro* or *in vivo*, which comprises administering to a patient in need thereof a tissue growth stimulating effective amount of at least one composition according to claim 38.

44. A method for modulating a TGFβ regulatable activity, comprising administering to a cell, cells, or a patient in need of such treatment, a composition according to claim 38.

45. A method for modulating a TGFβ regulatable activity, comprising administering to at least one cell, an organism, or a patient in need of such treatment, an antisense nucleic acid molecule having a nucleotide sequence complementary to at least 10 contiguous nucleotides of an mRNA transcribed from a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3, wherein said antisense nucleic acid molecule hybridizes to said contiguous sequence such that translation of said mRNA is inhibited.

46. A method for the prevention and/or treatment of a disease selected from the group of diseases consisting of cancer, fibrosis, osteoporosis, myocardial infarction, congestive heart failure, dilated cardiomyopathy, deep venous thrombosis, disseminated intravascular thrombosis, stroke, sepsis,

injuries involving major tissue damage and trauma, systemic inflammatory response syndrome, sepsis syndrome, septic shock, multiple organ dysfunction syndrome (including DIC), atherosclerotic plaque rupture, and associated sequela arising therefrom comprising administering to said patient at least one composition according to claim 38.

47. A method for the prevention and/or treatment of a disease selected from the group of diseases consisting of cancer, fibrosis, osteoporosis, myocardial infarction, congestive heart failure, dilated cardiomyopathy, deep venous thrombosis, disseminated intravascular thrombosis, stroke, sepsis, injuries involving major tissue damage and trauma, systemic inflammatory response syndrome, sepsis syndrome, septic shock, multiple organ dysfunction syndrome (including DIC), atherosclerotic plaque rupture, and associated sequela arising therefrom comprising administering to said patient at least one composition according to claim 39.

Respectfully submitted,

Robert L. Sharp
Attorney/Agent for Applicant
Registration No. 45,609
Phone: 317-276-5332

Eli Lilly and Company
Patent Division/RLS
Lilly Corporate Center
Indianapolis, Indiana 46285

February 27, 2001

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Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln
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Cys Leu Cys Pro Pro Asp Phe Thr Gly Arg Phe Cys Gln Val Pro Ala
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Gly Gly Ala Gly Gly Gly Thr Gly Gly Ser Gly Pro Gly Leu Ser Arg
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Thr Gly Ala Leu Ser Thr Gly Ala Leu Pro Pro Leu Ala Pro Glu Gly
115 120 125

Asp Ser Val Ala Ser Lys His Ala Ile Tyr Ala Val Gln Val Ile Ala
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Asp Pro Pro Gly Pro Gly Glu Gly Pro Pro Ala Gln His Ala Ala Phe
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Gly His Ser Thr Asp Thr Leu Thr Gly Ser Gly Phe Arg Val Val Val
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Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln
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Cys Leu Cys Pro Pro Asp Phe Thr Gly Arg Phe Cys Gln Val Pro Ala
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Gly Gly Ala Gly Gly Gly Thr Gly Gly Ser Gly Pro Gly Leu Ser Arg
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Thr Gly Ala Leu Ser Thr Gly Ala Leu Pro Pro Leu Ala Pro Glu Gly
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Asp Ser Val Ala Ser Lys His Ala Ile Tyr Ala Val Gln Val Ile Ala
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Asp Pro Pro Gly Pro Gly Glu Gly Pro Pro Ala Gln His Ala Ala Phe
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Leu Val Pro Leu Gly Pro Gly Gln Ile Ser Ala Glu Gly Thr Arg Gln
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Leu Ala Asn Pro Gly Arg Ser Pro Val Gly Gly His Xaa Gly Gly Gln
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Gly Arg Ala Gly Ser Ala Leu Glu Glu Leu Ser Ala Val Thr Arg Gly
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Ala Val Arg Ala Ala Leu Arg Pro Pro Arg Pro Pro Pro Val Gln Ala
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Pro Pro Pro Val Val Asn Val Arg Val His His Pro Pro Glu Ala Ser
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Ser Gln His Leu Leu Pro His Pro Lys Pro Ser His Pro Arg Pro Pro
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Thr Gln Lys Ser Leu Gly Arg Cys Phe Gln Asp Thr Leu Pro Lys Gln
275 280 285

Pro Cys Gly Ser Asn Pro Leu Pro Gly Leu Thr Lys Gln Glu Asp Cys
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Cys Gly Ser Ile Gly Thr Ala Trp Gly Gln Ser Lys Cys His Lys Cys
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Pro Gln Leu Gln Tyr Thr Gly Val Gln Lys Pro Gly Pro Val Arg Gly
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Glu Val Gly Ala Asp Cys Pro Gln Gly Tyr Lys Arg Leu Asn Ser Thr
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Gln His Pro Leu Thr Thr Arg Leu Thr Arg Gln Leu Cys Cys Cys Ser
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Val Gly Lys Ala Trp Gly Ala Arg Cys Gln Arg Cys Pro Thr Asp Gly
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Thr Ala Ala Phe Lys Glu Ile Cys Pro Ala Gly Lys Gly Tyr His Ile
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Leu Thr Ser His Gln Thr Leu Thr Ile Gln Gly Glu Ser Asp Phe Ser
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Leu Phe Leu His Pro Asp Gly Pro Pro Lys Pro Gln Gln Leu Pro Glu
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Ser Pro Ser Gln Ala Pro Pro Pro Glu Asp Thr Glu Glu Glu Arg Gly
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Val Thr Thr Asp Ser Pro Val Ser Glu Glu Arg Ser Val Gln Gln Ser
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His Pro Thr Ala Thr Thr Thr Pro Ala Arg Pro Tyr Pro Glu Leu Ile
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Ser Arg Pro Ser Pro Pro Thr Met Arg Trp Phe Leu Pro Asp Leu Pro
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Asp Glu Cys Arg Leu Asn Gln Asn Ile Cys Gly His Gly Glu Cys Val
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Pro Gly Pro Pro Asp Tyr Ser Cys His Cys Asn Pro Gly Tyr Arg Ser
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Asn Cys His Cys Asn Arg Gly Tyr Arg Leu His Val Gly Ala Gly Gly
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Arg Ser Cys Val Asp Leu Asn Glu Cys Ala Lys Pro His Leu Cys Gly
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Asp Gly Gly Phe Cys Ile Asn Phe Pro Gly His Tyr Lys Cys Asn Cys
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Tyr Pro Gly Tyr Arg Leu Lys Ala Ser Arg Pro Pro Val Cys Glu Asp
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Ile Asp Glu Cys Arg Asp Pro Ser Ser Cys Pro Asp Gly Lys Cys Glu
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Ser Gln Gly Gly Gly Ala Cys Arg Asp Val Asn Glu Cys Ala Glu Gly
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Cys Thr Cys Ala Gln Gly Tyr Ala Pro Ala Pro Asp Gly Arg Ser Cys
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Tyr Arg Cys Leu Cys Pro Gln Gly His Arg Leu Val Gly Gly Arg Lys
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Gln Pro His His Lys Lys Glu Cys Tyr Leu Asn Phe Asp Asp Thr Val
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Gly His Ser Thr Asp Thr Leu Thr Gly Ser Gly Phe Arg Val Val Val
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Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln
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180 185 190

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Gln His Leu Leu Pro His Pro Lys Pro Ser His Pro Arg Pro Pro Thr
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Gln Lys Ser Leu Gly Arg Cys Phe Gln Asp Thr Leu Pro Lys Gln Pro
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Gly Ser Ile Gly Thr Ala Trp Gly Gln Ser Lys Cys His Lys Cys Pro
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Gln Leu Gln Tyr Thr Gly Val Gln Lys Pro Gly Pro Val Arg Gly Glu
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Val Gly Ala Asp Cys Pro Gln Gly Tyr Lys Arg Leu Asn Ser Thr His
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Cys Gln Asp Ile Asn Glu Cys Ala Met Pro Gly Val Cys Arg His Gly
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Asp Cys Leu Asn Asn Pro Gly Ser Tyr Arg Cys Val Cys Pro Pro Gly
325 330 335

His Ser Leu Gly Pro Ser Arg Thr Gln Cys Ile Ala Asp Lys Pro Glu
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Glu Lys Ser Leu Cys Phe Arg Leu Val Ser Pro Glu His Gln Cys Gln
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His Pro Leu Thr Thr Arg Leu Thr Arg Gln Leu Cys Cys Cys Ser Val
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Gly Lys Ala Trp Gly Ala Arg Cys Gln Arg Cys Pro Thr Asp Gly Thr
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Ala Ala Phe Lys Glu Ile Cys Pro Ala Gly Lys Gly Tyr His Ile Leu
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Thr Ser His Gln Thr Leu Thr Ile Gln Gly Glu Ser Asp Phe Ser Leu
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Phe Leu His Pro Asp Gly Pro Pro Lys Pro Gln Gln Leu Pro Glu Ser
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Pro Ser Gln Ala Pro Pro Pro Glu Asp Thr Glu Glu Glu Arg Gly Val
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Thr Thr Asp Ser Pro Val Ser Glu Glu Arg Ser Val Gln Gln Ser His
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Pro Thr Ala Thr Thr Thr Pro Ala Arg Pro Tyr Pro Glu Leu Ile Ser
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Arg Pro Ser Pro Pro Thr Met Arg Trp Phe Leu Pro Asp Leu Pro Pro
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Ser Arg Ser Ala Val Glu Ile Ala Pro Thr Gln Val Thr Glu Thr Asp
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Glu Cys Arg Leu Asn Gln Asn Ile Cys Gly His Gly Glu Cys Val Pro
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Gly Pro Pro Asp Tyr Ser Cys His Cys Asn Pro Gly Tyr Arg Ser His
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Pro Gln His Arg Tyr Cys Val Asp Val Asn Glu Cys Glu Ala Glu Pro
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Cys Gly Pro Gly Arg Gly Ile Cys Met Asn Thr Gly Gly Ser Tyr Asn
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Cys His Cys Asn Arg Gly Tyr Arg Leu His Val Gly Ala Gly Gly Arg
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            820                 825                 830

Gly Ala Cys Lys Asn Leu Gln Gly Ser Tyr Val Cys Val Cys Asp Glu
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Gly Phe Thr Pro Thr Gln Asp Gln His Gly Cys Glu Glu Val Glu Gln
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Pro His His Lys Lys Glu Cys Tyr Leu Asn Phe Asp Asp Thr Val Phe
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Cys Asp Ser Val Leu Ala Thr Asn Val Thr Gln Gln Glu Cys Cys Cys
                885                 890                 895

Ser Leu Gly Ala Gly Trp Gly Asp His Cys Glu Ile Tyr Pro Cys Pro
            900                 905                 910

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Ser His Cys Pro Thr Ser Gln Ser Glu Ser Asn Ser Phe Trp Asp Thr
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Ser Pro Leu Leu Leu Gly Lys Pro Pro Arg Asp Glu Asp Ser Ser Glu
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Pro Gly Gly Ala Val Cys Glu Cys Pro Gly Gly Phe Gln Leu Asp Ala
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Ser Arg Ala Arg Cys Val Asp Ile Asp Glu Cys Arg Glu Leu Asn Gln
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Arg Gly Leu Leu Cys Lys Ser Glu Arg Cys Val Asn Thr Ser Gly Ser
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Gly His Ser Thr Asp Thr Leu Thr Gly Ser Gly Phe Arg Val Val Val
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Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln
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Cys Leu Cys Pro Pro Asp Phe Thr Gly Arg Phe Cys Gln Val Pro Ala
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Gly His Ser Thr Asp Thr Leu Thr Gly Ser Gly Phe Arg Val Val Val

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Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln
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Cys Leu Cys Pro Pro Asp Phe Thr Gly Arg Phe Cys Gln Val Pro Ala
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Gly Gly Ala Gly Gly Gly Thr Gly Gly Ser Gly Pro Gly Leu Ser Arg
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Thr Gly Ala Leu Ser Thr Gly Ala Leu Pro Pro Leu Ala Pro Glu Gly
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Leu Ala Asn Pro Gly Arg Ser Pro Val Gly Gly His Xaa Gly Gly Gln
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Ala Val Arg Ala Ala Leu Arg Pro Pro Arg Pro Pro Pro Val Gln Ala
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Pro Pro Pro Val Val Asn Val Arg Val His His Pro Pro Glu Ala Ser
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Val Gln Val His Arg Ile Glu Ser Ser Asn Ala Glu Ser Ala Ala Pro
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Ser Gln His Leu Leu Pro His Pro Lys Pro Ser His Pro Arg Pro Pro
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Thr Gln Lys Ser Leu Gly Arg Cys Phe Gln Asp Thr Leu Pro Lys Gln
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Pro Cys Gly Ser Asn Pro Leu Pro Gly Leu Thr Lys Gln Glu Asp Cys
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Cys Gly Ser Ile Gly Thr Ala Trp Gly Gln Ser Lys Cys His Lys Cys
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Glu Val Gly Ala Asp Cys Pro Gln Gly Tyr Lys Arg Leu Asn Ser Thr
340 345 350

His Cys Gln Asp Ile Asn Glu Cys Ala Met Pro Gly Val Cys Arg His
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Gly Asp Cys Leu Asn Asn Pro Gly Ser Tyr Arg Cys Val Cys Pro Pro
370 375 380

Gly His Ser Leu Gly Pro Ser Arg Thr Gln Cys Ile Ala Asp Lys Pro
385 390 395 400

Glu Glu Lys Ser Leu Cys Phe Arg Leu Val Ser Pro Glu His Gln Cys
405 410 415

Gln His Pro Leu Thr Thr Arg Leu Thr Arg Gln Leu Cys Cys Cys Ser
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Val Gly Lys Ala Trp Gly Ala Arg Cys Gln Arg Cys Pro Thr Asp Gly
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Thr Ala Ala Phe Lys Glu Ile Cys Pro Ala Gly Lys Gly Tyr His Ile
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465 470 475 480

Leu Phe Leu His Pro Asp Gly Pro Pro Lys Pro Gln Gln Leu Pro Glu
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Val Thr Thr Asp Ser Pro Val Ser Glu Glu Arg Ser Val Gln Gln Ser
515 520 525

His Pro Thr Ala Thr Thr Thr Pro Ala Arg Pro Tyr Pro Glu Leu Ile
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580 585 590

Pro Gly Pro Pro Asp Tyr Ser Cys His Cys Asn Pro Gly Tyr Arg Ser
595 600 605

His Pro Gln His Arg Tyr Cys Val Asp Val Asn Glu Cys Glu Ala Glu
610 615 620

Pro Cys Gly Pro Gly Arg Gly Ile Cys Met Asn Thr Gly Gly Ser Tyr
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Asn Cys His Cys Asn Arg Gly Tyr Arg Leu His Val Gly Ala Gly Gly
645 650 655

Arg Ser Cys Val Asp Leu Asn Glu Cys Ala Lys Pro His Leu Cys Gly
660 665 670

Asp Gly Gly Phe Cys Ile Asn Phe Pro Gly His Tyr Lys Cys Asn Cys
675 680 685

Tyr Pro Gly Tyr Arg Leu Lys Ala Ser Arg Pro Pro Val Cys Glu Asp
690 695 700

Ile Asp Glu Cys Arg Asp Pro Ser Ser Cys Pro Asp Gly Lys Cys Glu
705 710 715 720

Asn Lys Pro Gly Ser Phe Lys Cys Ile Ala Cys Gln Pro Gly Tyr Arg
725 730 735

Ser Gln Gly Gly Gly Ala Cys Arg Asp Val Asn Glu Cys Ala Glu Gly
740 745 750

Ser Pro Cys Ser Pro Gly Trp Cys Glu Asn Leu Pro Gly Ser Phe Arg
755 760 765

Cys Thr Cys Ala Gln Gly Tyr Ala Pro Ala Pro Asp Gly Arg Ser Cys
770 775 780

Leu Asp Val Asp Glu Cys Glu Ala Gly Asp Val Cys Asp Asn Gly Ile
785 790 795 800

Cys Ser Asn Thr Pro Gly Ser Phe Gln Cys Gln Cys Leu Ser Gly Tyr
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His Leu Ser Arg Asp Arg Ser His Cys Glu Asp Ile Asp Glu Cys Asp
820 825 830

Phe Pro Ala Ala Cys Ile Gly Gly Asp Cys Ile Asn Thr Asn Gly Ser
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Tyr Arg Cys Leu Cys Pro Gln Gly His Arg Leu Val Gly Gly Arg Lys
850 855 860

Cys Gln Asp Ile Asp Glu Cys Ser Gln Asp Pro Ser Leu Cys Leu Pro
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His Gly Ala Cys Lys Asn Leu Gln Gly Ser Tyr Val Cys Val Cys Asp
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Glu Gly Phe Thr Pro Thr Gln Asp Gln His Gly Cys Glu Glu Val Glu
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Gln Pro His His Lys Lys Glu Cys Tyr Leu Asn Phe Asp Asp Thr Val
915 920 925

Phe Cys Asp Ser Val Leu Ala Thr Asn Val Thr Gln Gln Glu Cys Cys
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Cys Ser Leu Gly Ala Gly Trp Gly Asp His Cys Glu Ile Tyr Pro Cys
945 950 955 960

Pro Val Tyr Ser Ser Ala Glu Phe His Ser Leu Cys Pro Asp Gly Lys
965 970 975

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1235 1240 1245

Gly Ala Cys Val Pro Gln Arg Arg Arg
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HUMAN LATENT TRANSFORMING GROWTH FACTOR-β BINDING PROTEIN 3

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to recombinant DNA technology. In particular the invention pertains to genes and proteins that are involved in the regulation of TGF-β activity, methods for making and using same, and pharmaceutical compositions thereof.

RELATED ART

The transforming growth factor-β (TGF-β) superfamily of cytokines comprises a large number of secreted growth and differentiation factors that play important roles in embryonic development, cellular proliferation, and tissue homeostasis. The superfamily includes, for instance, the TGF-βs, the growth and differentiation factors (GDFs), the activins, and the bone morphogenetic proteins (BMPs) among others (for review see Kingsley, *Genes and Development* 8:133-146, 1994). Making up the TGF-β sub-family are five related proteins, TGF-β1, TGF-β2, TGF-β3, TGF-β4, and TGF-β5 having 70-80% sequence identity among themselves. Each of these proteins can affect diverse cellular functions in virtually all cell types. TGF-β1, for instance, has been shown to inhibit the proliferation of endothelial cells (Mueller, G.J. et al., *Proc. Nat. Acad. Sci.*, 84:5600-5604 (1987)), and to stimulate the proliferation of fibroblasts under certain conditions (Barnard, J.A. et al., *Biochem. Biophys. Res. Commun.*, 163:56-63, (1997)).

The TGF-βs are initially synthesized as large precursor molecules consisting mostly of disulfide-linked homodimers. During biosynthesis the mature TGF-β dimer is proteolytically cleaved and secreted as a small latent

complex consisting of a biologically inactive, noncovalently-bound complex of dimers of the amino terminal precursor remainder, designated latency associated peptide (LAP), and mature TGF-β (Miyazono *et* al., *J. Biol. Chem.* 263:6407-6415 (1988)). These small latent complexes are often associated with a large molecular weight binding protein called a latent TGF-β binding protein (LTBP).

The LTBP gene family consists of three members, LTBP-1, LTBP-2, and LTBP-3, having cysteine rich multidomain structures and multiple (8-20) epidermal growth factor (EGF)-like repeats in a contiguous segment. EGF motifs are commonly found in extracellular matrix proteins where they are thought to mediate high-affinity calcium binding and to provide structural stability to regions that connect other functional domains. The eight-cysteine motifs, however, are unique to the fibrillin and LTBP gene families.

There is convincing evidence that the secreted LTBP proteins bind latent growth factors, namely TGF-βs, intracellularly and facilitate their folding and secretion to proper extracellular matrix storage sites. It has also been suggested that LTBPs protect the small latent complexes from proteolytic activity which governs the activation of latent complexes (Wushan, Y. et al., *J. Bio. Chem.* 270:10147-10160, (1995)).

There is also much data to suggest that dysregulated production and activation of TGF-β is a contributing factor in fibrotic disease (Broekelmann et al., *Proc. Nat. Acad. Sci. USA,* 88:6642-6646, (1991) and Khalil et al., *J. Exp. Med.,* 170:727-737, (1989)) and tumorigenesis, as well as conditions such as human diabetic nephropathy (Hoffman et al., *Miner. Electrolyte Metab.,* 24:190-196, (1998)), bone resorption, bone formation, and cartilage formation.

BRIEF SUMMARY OF THE INVENTION

The present invention provides novel human TGF-β latent binding protein-related nucleic acid molecules, their encoded polypeptides, pharmaceutical compositions comprising same, and therapeutic uses thereof. Nucleic acids and polypeptides of the invention are referred to herein as hLTBP-3. SEQ ID NOS: 1 and 3 originate from human sources and are useful, among other things, as probes to isolate paralogous genes from humans as well as orthologous genes from other organisms.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising a polynucleotide encoding specific hLTBP-3 polypeptides, hLTBP-3 polypeptide fragments, as well as variants comprising at least one domain thereof. Such polypeptides are provided as non-limiting examples by the corresponding domains, fragments and/or variants as hLTBP-3 polypeptides corresponding to at least five amino acid fragments of SEQ ID NOS: 2, 4, or 6. The hLTBP-3 polypeptides provide multiple utilities, for example, as expected pharmaceutical compounds for inhibiting the fibrotic process, treating tumors, inhibiting bone resorption, and stimulating bone and/or cartilage formation.

Furthermore, the current invention provides in one aspect a method for treating or preventing a disease in which TGFβ is responsible for inducing cellular effects that lead to at least one disease state; the method comprising administering to a patient in need of such treatment, a compound that modulates the activity of TGFβ.

The current invention also provides methods for treating or preventing at least one aspect of at least one vascular disease; the method comprising administering to a patient in need of such treatment, a polypeptide of the present invention that modulates at least one TGFβ regulated activity. Preferred TGFβ regulatable activities include, but are not limited to, plasminogen activator inhibitor-1 (PAI-1) expression and/or

activity, PAI-1 secretion, thrombomodulin expression and/or activity, TGFβ secretion, and cell proliferation.

As part of the current invention, there is provided methods for identifying compounds, such as polypeptides or organic molecules, that modulate the activity of TGFβ (e.g., proteins or polypeptides encoded by SEQ ID NO:1 and 3, respectively), which in turn modulate the expression or activity of anticoagulant and fibrinolytic functions, namely PAI-I and TM, in the vasculature endothelium.

In another embodiment the present invention relates to a substantially pure polypeptide comprising the amino acid sequence which is SEQ ID NO: 2, 4, or SEQ ID NO: 6.

The present invention also provides a method for modulating TGFβ regulatable activities comprising administering to a patient in need of such treatment, a protein product encoded by a nucleic acid molecule having at least 70, 75, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to a member selected from the group consisting of at least 30 nucleotides of at least one of SEQ ID NO:1, SEQ ID NO:3, and a nucleic acid molecule that is complementary to SEQ ID NO:1 or SEQ ID NO:3. Preferred protein products for use in the current invention include SEQ ID NOS:2,4, and 6. A nucleic acid molecule having at least 70% identity to the specified sequences are preferred for use in the present invention; identity of at least 95% is especially preferred. Preferred TGFβ regulatable activities include, but are not limited to, PAI-1 expression and/or activity, PAI-1 secretion, thrombomodulin expression and/or activity, TGFβ secretion, and cell proliferation.

The present invention also provides a method for modulating TGFβ regulatable activities comprising administering to a cell or cells, a protein product encoded by a nucleic acid molecule having at least 70, 75, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or

100% identity to a member selected from the group consisting of at least 30 nucleotides of at least one of SEQ ID NO:1, SEQ ID NO:3, or a nucleic acid molecule that is complementary to SEQ ID NO:1 or SEQ ID NO:3. Preferred protein products for use in the current invention include those as shown in SEQ ID NOS:2, 4, 6. A nucleic acid molecule having at least 70% identity to the specified sequences are preferred for use in the present invention; identity of at least 95% is especially preferred. Preferred TGFβ regulatable activities include, but are not limited to, PAI-1 expression and/or activity, PAI-1 secretion, thrombomodulin expression and/or activity, TGFβ secretion, and cell proliferation.

In another embodiment the present invention relates to an isolated nucleic acid compound encoding an hLTBP-3 polypeptide. Preferably, the present invention relates to an isolated nucleic acid compound that encodes a polypeptide of the invention said nucleic acid being as shown in SEQ ID NOS: 1 or 3.

The current invention also provides a method for modulating TGFβ regulatable activities comprising administering to at least one cell, an organism, or a patient in need of such treatment, an antisense nucleic acid molecule having a nucleotide sequence complementary to a contiguous sequence of mRNA transcribed from a gene selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3, wherein said antisense nucleic acid molecule hybridizes to said contiguous sequence such that translation of said mRNA is inhibited. It is preferred if the contiguous sequence includes at least fifteen nucleotides. Preferred nucleic acid molecules for use in the current invention include complementary sequences to the polynucleotides as shown in SEQ ID NO:1 or SEQ ID NO:3, as well as fragments thereof. Preferred TGFβ regulatable activities include, but are not limited to, PAI-1 expression

and/or activity, PAI-1 secretion, thrombomodulin expression and/or activity, TGFβ secretion, and cell proliferation.

The invention also provides methods for the identification of compounds that modulate a TGFβ regulatable activity comprising administering to at least one cell or an organism a compound that modulates expression of, or the activity of the protein product of, a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and fragments thereof. Preferred TGFβ regulatable activities include, but are not limited to, PAI-1 expression and/or activity, PAI-1 secretion, thrombomodulin expression and/or activity, TGFβ secretion, and cell proliferation.

In another embodiment the present invention relates to an isolated nucleic acid molecule useful as a hybridization probe comprising SEQ ID NO: 1, SEQ ID NO: 3, or fragments thereof consisting of at least 14 contiguous base pairs.

In another embodiment the present invention relates to an isolated nucleic acid compound that encodes a polypeptide having the ability to induce or inhibit cellular proliferation wherein said nucleic acid hybridizes to SEQ ID NOS: 1 or 3 under high stringency conditions.

In another embodiment the present invention relates to a vector comprising an isolated hLTBP-3 nucleic acid compound as shown in SEQ ID NOS: 1 or 3, as well as fragments thereof.

In another embodiment the present invention relates to a vector wherein said isolated nucleic acid compound is operably-linked to a promoter sequence.

In another embodiment the present invention relates to a host cell containing a vector of the present invention.

In another embodiment the present invention relates to a method for constructing a recombinant host cell having the potential to express an hLTBP-3 polypeptide, said method comprising introducing into said host cell by any suitable means a vector of the present invention.

In another embodiment the present invention relates to a method for expressing an hLTBP-3 polypeptide in a recombinant host cell, said method comprising culturing said recombinant host cell under conditions suitable for gene expression.

In another embodiment the present invention relates to a method for identifying compounds that bind a polypeptide having the translated amino acid sequence selected from the group consisting SEQ ID NOS: 2, 4, and 6 wherein said method comprises the steps of:

a) admixing a substantially purified preparation of a polypeptide identified herein with a test compound; and

b) monitoring by any suitable means a binding interaction between said polypeptide and said compound.

In still another embodiment the present invention relates to an antibody that selectively binds a polypeptide having the translated amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, and 6.

In another embodiment the present invention relates to a pharmaceutical formulation comprising as an active ingredient an hLTBP-3 polypeptide, associated with one or more pharmaceutically acceptable carriers, excipients, or diluents thereof.

In another embodiment the present invention relates to a method for modulating tissue growth by administration of a therapeutically effective amount of hLTBP-3.

In still another embodiment the present invention relates to a method for inhibiting tumor growth by administration of a therapeutically effective amount of hLTBP-3.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab_2', and Fv fragments), and

chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived.

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding to form double stranded nucleic acid molecules. The following base pairs are related by complementarity: guanine and cytosine; adenine and thymine; and adenine and uracil. As used herein, "complementary" means that the aforementioned relationship applies to substantially all base pairs comprising two single-stranded nucleic acid molecules over the entire length of said molecules. "Partially complementary" refers to the aforementioned relationship in which one of two single-stranded nucleic acid molecules is shorter in length than the other such that a portion of one of the molecules remains single-stranded.

The term "conservative substitution" or "conservative amino acid substitution" refers to a replacement of one or more amino acid residue(s) in a parent polypeptide as stipulated by Table 1.

The term "fragment thereof" refers to a fragment, piece, or sub-region of a nucleic acid or polypeptide molecule whose sequence is disclosed herein, such that the fragment comprises 5 or more amino acids, or 10 or more nucleotides that are contiguous in the parent polypeptide or nucleic acid molecule. "Fragment thereof" may or may not retain biological activity. A fragment of a polypeptide disclosed herein could be used as an antigen to raise a specific antibody against the parent polypeptide molecule. With reference to a nucleic acid molecule, "fragment thereof" refers to 10 or more contiguous nucleotides, derived from a parent nucleic acid. The term also encompasses the complementary sequence. For example if the fragment entails the sequence 5'-AGCTAG-3', then "fragment thereof" would also include the complementary sequence, 3'-TCGATC-5'.

The term "functional fragment" or "functionally equivalent fragment", as used herein, refers to a region, or fragment of a full length polypeptide, or sequence of amino acids that, for example, comprises an active site, or any other motif, relating to biological function. Functional fragments are capable of providing a substantially similar biological activity as a polypeptide disclosed herein, *in vivo* or *in vitro*, *viz.* the capacity to modulate cell proliferation. Functional fragments may be produced by cloning technology, or as the natural products of alternative splicing mechanisms.

The term "functionally related" is used herein to describe polypeptides that are related to the hLTBP-3 polypeptides of the present invention, said functionally related polypeptides constituting modifications of said hLTBP-3 polypeptides, in which conservative amino acid changes are present as natural polymorphic variants of the polypeptides disclosed herein. Conservative amino acid substitutions and modifications may also be engineered using by recombinant DNA techniques. Functionally related polypeptides retain the biological activity of hLTBP-3, such as the ability to inhibit cell proliferation, and/or tumor growth in vivo or in vitro.

The term "host cell" refers to any eucaryotic or procaryotic cell that is suitable for propagating and/or expressing a cloned gene contained on a vector that is introduced into said host cell by, for example, transformation or transfection, or the like.

The term "homolog" or "homologous" describes the relationship between different nucleic acid molecules, or amino acid sequences, in which said sequences or molecules are related by partial identity or chemical/physical similarity at one or more blocks or regions within said molecules or sequences.

The term "hybridization" as used herein refers to a process in which a single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base

pairing. "Selective hybridization" refers to hybridization under conditions of high stringency. The degree of hybridization depends upon, for example, the degree of homology, the stringency of hybridization, and the length of hybridizing strands.

The term "isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

The term "mature protein" or "mature polypeptide" as used herein refers to the form(s) of the protein produced by expression in a mammalian cell. It is generally hypothesized that once export of a growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal peptide (SP) sequence which is cleaved from the complete polypeptide to produce a "mature" form of the protein. Oftentimes, cleavage of a secreted protein is not uniform and may result in more than one species of mature protein. The cleavage site of a secreted protein is determined by the primary amino acid sequence of the complete protein and generally can not be predicted with complete accuracy. However, cleavage sites for a secreted protein may be determined experimentally by amino-terminal sequencing of the one or more species of mature proteins found within a purified preparation of the protein. Furthermore, one skilled in the art of genetic engineering can construct DNA sequences encoding a putative mature protein species fused to a heterologous SP (e.g., SEQ ID NO: 5) in order to optimize secretion and/or tissue distribution of the expressed protein product.

The symbol "N" in a nucleic acid sequence refers to adenine ("A"), guanine ("G"), cytosine ("C"), thymine (T"), or uracil ("U"); "Z" designates an unknown amino acid residue; "Xaa" designates any of the known amino acids.

The term "nucleic acid probe" or "probe" as used herein is a labeled nucleic acid compound which hybridizes

with another nucleic acid compound. "Nucleic acid probe" means a single stranded nucleic acid sequence that will combine with a complementary or partially complementary single stranded target nucleic acid sequence to form a double-stranded molecule. A nucleic acid probe may be an oligonucleotide or a nucleotide polymer. A probe will usually contain a detectable moiety which may be attached to the end(s) of the probe or be internal to the sequence of the probe.

The term "orthologue" or "orthologous" refers to two or more genes or polypeptides from different organisms that exhibit sequence homology.

The term "paralogue" or "paralogous" refers to two or more genes or polypeptides within a single organism that exhibit sequence homology.

The term "plasmid" refers to an extrachromosomal genetic element. The plasmids disclosed herein are commercially available, publicly available on an unrestricted basis, or can be constructed from readily available plasmids in accordance with published procedures.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule.

The term "promoter" refers to a nucleic acid sequence that directs transcription, for example, of DNA to RNA. An inducible promoter is one that is regulatable by environmental signals, such as carbon source, heat, or metal ions, for example. A constitutive promoter generally operates at a constant level and is not regulatable.

The terms "protein" and "polypeptide" are used interchangeably herein and are intended to mean a biopolymer comprising a plurality of amino acid residues covalently bound in peptide linkage.

The term "recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages,

comprising a DNA molecule to which one or more additional DNA segments can or have been incorporated.

The term "recombinant DNA expression vector" or "expression vector" as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present thereby enabling transcription of an inserted DNA, which may encode a protein.

The term "stringency" refers to hybridization conditions. High stringency conditions disfavor non-homologous base pairing. Low stringency conditions have the opposite effect. Stringency may be altered, for example, by temperature and salt concentration.

The term "low stringency" refers to conditions that comprise, for example, a temperature of about 37° C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50° C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

The term "high stringency" refers to conditions that comprise, for example, a temperature of about 42° C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65° C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. *et al.* Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

The term "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

The term "SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na_2HPO_4, 0.9 mM NaH_2PO_4 and 1 mM EDTA, pH 7.4.

The term "substantially pure," used in reference to a polypeptide, means substantial separation from other cellular and non-cellular molecules, including other protein molecules. A substantially pure preparation would be about at least 85% pure; preferably about at least 95% pure. A "substantially pure" protein can be prepared by a variety of techniques, well known to the skilled artisan, including, for example, the IMAC protein purification method.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous DNA into host cells. A vector comprises a nucleotide sequence which may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

The various restriction enzymes disclosed and described herein are commercially available and the manner of use of said enzymes including reaction conditions, cofactors, and other requirements for activity are well known to one of ordinary skill in the art. Reaction conditions for particular enzymes are carried out according to the manufacturer's recommendation.

The hLTBP-3 gene of the present invention encodes a protein that is the human homolog of the mouse latent transforming growth factor-β binding protein-3 gene. (*See e.g.* Wushan, Y. et al., *J. Bio. Chem.* 270:10147-10160, (1995)). These proteins are often co-expressed with TGF-βs and appear to modulate the activation process of TGF-βs. Thus, the hLTBP genes and their protein products are useful for modulating the activity of TGFβ. For instance, the hLTBP-3 nucleic acids and the polypeptides they encode can be used to inhibit cell proliferation where such would be beneficial, for example, in treating tumors or cancers. The

hLTBP-3 gene of this invention is expressed in numerous tissues including brain, kidney, cartilage, ovary, pancreas, stomach, and spleen.

An embodiment of a hLTBP-3 DNA sequence is disclosed herein by SEQ ID NOS:1 and 3. Those skilled in the art will recognize that owing to the degeneracy of the genetic code, numerous "silent" substitutions of nucleotide base pairs could be introduced into the sequences identified herein without altering the identity of the encoded amino acid(s) or protein or protein product. All such substitutions are intended to be within the scope of the invention.

The invention further provides isolated hLTBP-3 polypeptides, as well as fragments or specified variants thereof, comprising the amino acid sequence encoded by the deposited cDNAs, or the amino acid sequences in SEQ ID NOS:2, 4, or 6.

The isolated proteins of the present invention comprise a polypeptide having at least 5 - 10 amino acids encoded by any one of the polynucleotides of the present invention as discussed more fully, supra, or polypeptides which are conservatively modified variants thereof.

Exemplary polypeptide sequences are provided in SEQ ID NOS:2, 4, and 6. The proteins of the present invention or variants thereof can comprise any number of contiguous amino acid residues from a polypeptide of the present invention, wherein that number is selected from the group of integers consisting of from 10 to the number of residues in a full-length hLTBP-3 polypeptide. Optionally, this subsequence of contiguous amino acids is at least 15, 20, 25, 30, 35, or 40 amino acids in length, often at least 50, 60, 70, 80, or 90 amino acids in length. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes biologically active polypeptides of the present

invention (i.e., enzymes). Biologically active polypeptides have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95% - 100% of that of the native (non-synthetic), endogenous polypeptide. Further, the substrate specificity (k_{cat}/K_m) is optionally substantially similar to the native (non-synthetic), endogenous polypeptide. Typically, the K_m will be at least 30%, 40%, or 50%, that of the native (non-synthetic), endogenous polypeptide; and more preferably at least 60%, 70%, 80%, or 90%. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity (k_{cat}/K_m), are well known to those of skill in the art.

Generally, the polypeptides of the present invention will, when presented as an immunogen, elicit production of an antibody specifically reactive to a polypeptide of the present invention encoded by a polynucleotide of the present invention as described, supra. Further, the proteins of the present invention will not bind to antisera raised against a polypeptide of the present invention which has been fully immunosorbed with the same polypeptide. Immunoassays for determining binding are well known to those of skill in the art. A preferred immunoassay is a competitive immunoassay as discussed, infra. Thus, the proteins of the present invention can be employed as immunogens for constructing antibodies immunoreactive to a protein of the present invention for such exemplary utilities as immunoassays or protein purification techniques.

A hLTBP-3 polypeptide of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein. Therefore, contemplated within the scope of the present invention are hLTBP-3 polypeptides with or without amino acid sequences comprising amino acid residues 180-228 of SEQ ID NO: 6 and/or 1-41 of

SEQ ID NO: 5 or fragments thereof.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions for any given hLTBP-3 polypeptide will not be more than 20, 10, 5, or 3, such as 1-20 or any range or value therein, as specified herein.

Amino acids in a hLTBP-3 polypeptide of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity. Sites that are critical for ligand-protein binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., J. Mol. Biol. 224:899-904 (1992) and de Vos et al. Science 255:306-312 (1992)).

hLTBP-3 polypeptides of the present invention can include, but are not limited to, at least one domain selected from the signal peptide and active site domains of SEQ ID NOS:2, 4, 5, or 6.

A hLTBP-3 polypeptide can further comprise a polypeptide encoded by 1-2000 contiguous amino acids of SEQ ID NOS:2, 4, or 6.

A hLTBP-3 polypeptide can further comprise a polypeptide encoded by 1-2000 contiguous amino acids of SEQ ID NOS:2, 4, or 6 wherein said polypeptide is fused to a signal peptide including, but not limited to, the signal peptide shown as SEQ ID NO:5.

Non-limiting mutants that can enhance, decrease, or maintain at least one of the listed activities include, but are not limited to, any of the above polypeptides, further comprising at least one mutation corresponding to at least one substitution selected from the group consisting of 66A,

104A, 106T, 108A, 112S, 117W, 118P, 120D, 121R, 123M, 127P, 136E, 225P, 234P, 290V, 323N, 324V, 325C, 349L, 350A, 351A, 370T, 404A, 458R, 462L, 473M, 475P, 493S, 495P, 548Q, 554S, 562A, 588K, 659I, 671T, 708S, 711T, 725Y, 760T, 782R, 813L, 930V, 957I, 961A, 1084T, 1094T, 1097Q, 1119S, and 1201T corresponding to the amino acids as presented in SEQ ID NO: 2 or selected from the group consisting of 274P, 283P, 339V, 372N, 373V, 374C, 398L, 399A, 400A, 419T, 453A, 507R, 511L, 522M, 524P, 542S, 544P, 597Q, 603S, 611A, 637K, 708I, 720T, 757S, 760T, 774Y, 809T, 831R, 862L, 979V, 1,006I, 1010A, 1133T, 1143T, 1146Q, 1168S, and 1250T corresponding to the amino acids as presented in SEQ ID NO: 6.

Also contemplated by the present invention are proteins that are functionally related to hLTBP-3. For example, proteins that are functionally related to SEQ ID NO: 2,4, or 6 may be produced by conservative amino acid substitutions, replacements, deletions, or insertions, at one or more amino acid positions within hLTBP-3, in accordance with the Table presented herein.

Modifications of hLTBP-3 polypeptides made in accordance with the Table are generally expected to retain the biological activity of the parent molecule based on art recognized substitutability of the amino acids specified in the Table (*See e.g.* M. Dayhoff, In Atlas of Protein Sequence and Structure, Vol. 5, Supp. 3, pgs 345-352, 1978). hLTBP-3 functionality is easily tested, for example, in an assay that measures the ability to inhibit cell proliferation.

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS
ALA	SER, THR
ARG	LYS
ASN	HIS, SER
ASP	GLU, ASN
CYS	SER
GLN	ASN, HIS
GLU	ASP, GLU
GLY	ALA, SER
HIS	ASN, GLN
ILE	LEU, VAL, THR
LEU	ILE, VAL
LYS	ARG, GLN, GLU, THR
MET	LEU, ILE, VAL
PHE	LEU, TYR
SER	THR, ALA, ASN
THR	SER, ALA
TRP	ARG, SER
TYR	PHE
VAL	ILE, LEU, ALA
PRO	ALA

Fragments of proteins

One embodiment of the instant invention provides fragments of the hLTBP-3 polypeptide that may or may not be biologically active. Such fragments are useful, for example, as an antigen for producing an antibody to said protein.

Fragments of the protein disclosed herein may be generated by any number of suitable techniques, including chemical synthesis of any portion of hLTBP-3 (e.g. SEQ ID NOS: 2, 4, 5, or 6), proteolytic digestion of said proteins, or most preferably, by recombinant DNA mutagenesis techniques, well known to the skilled artisan. *See. e.g.* K. Struhl, "Reverse biochemistry: Methods and applications for synthesizing yeast proteins *in vitro*," *Meth. Enzymol.* 194,

520-535. For example, in a preferred method, a nested set of deletion mutations are introduced into a gene encoding hLTBP-3 (e.g. SEQ ID NOS: 1 or 3), or gene fragment thereof such that varying amounts of the protein coding region are deleted, either from the amino terminal end, or from the carboxyl end of the protein molecule. This method can also be used to create internal fragments of the intact protein in which both carboxyl and amino terminal ends are removed. Several appropriate nucleases can be used to create such deletions, for example *Bal*31, or in the case of a single stranded nucleic acid molecule, mung bean nuclease. For simplicity, it is preferred that the hLTBP-3 gene be cloned into a single-stranded cloning vector, such as bacteriophage M13, or equivalent. If desired, the resulting deletion fragments can be subcloned into any suitable vector for propagation and expression in any suitable host cell.

Functional fragments of the hLTBP-3 polypeptide of this invention may be produced as described above, preferably using cloning techniques to engineer smaller versions of the hLTBP-3 gene, lacking sequence from the 5' end, the 3' end, from both ends, or from an internal site. Smaller fragments of the genes or gene fragments of this invention can be used as a template to produce the encoded proteins. Biological function can be tested using any suitable assay to measure, for example, the ability to modify cell proliferation *in vivo* or *in vitro*. For example, hLTBP-3 can be used to block the TGF-β-mediated inhibition of Mv1Lu cells or primary hepatocyte growth in vitro as monitored by ^{3}H-thymidine uptake, essentially as described in Bottinger, E. P. et al., *Proc, Nat. Acad. Sci.*, 93: 5877-5882 (1996).

Gene Isolation Procedures

Those skilled in the art will recognize that the hLTBP-3 gene could be obtained by a plurality of recombinant DNA techniques including, for example, hybridization, polymerase

chain reaction (PCR) amplification, or *de novo* DNA synthesis. (*See e.g.*, T. Maniatis *et al.* Molecular Cloning: A Laboratory Manual, 2d Ed. Chap. 18 (1989)).

Methods for constructing cDNA libraries in a suitable vector such as a plasmid or phage for propagation in procaryotic or eucaryotic cells are well known to those skilled in the art. [*See e.g.* Maniatis *et al. Supra*]. Suitable cloning vectors are well known and are widely available.

The hLTBP-3 gene, or fragments thereof, can be isolated from any tissue in which said gene is expressed. For example, hLTBP-3 (SEQ ID NOS: 2 or 6) is expressed in at least one of the following tissues including brain, kidney, and spleen.

In one method for gene isolation, mRNA is isolated from a suitable tissue that expresses hLTBP-3, and first strand cDNA synthesis is carried out. Second round DNA synthesis can be carried out for the production of the second strand. If desired, the double-stranded cDNA can be cloned into any suitable vector, for example, a plasmid, thereby forming a cDNA library. Oligonucleotide primers targeted to any suitable region of the sequences disclosed herein can be used for PCR amplification of hLTBP-3 genes. *See e.g.* PCR Protocols: A Guide to Method and Application, Ed. M. Innis et al., Academic Press (1990). The PCR amplification comprises template DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Protein Production Methods

One embodiment of the present invention relates to substantially purified proteins encoded by the hLTBP-3 genes.

Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of

different methods, such as chemical methods well known in the art, including solid phase protein synthesis or recombinant methods. Both methods are described in U.S. Patent 4,617,189, incorporated herein by reference.

The principles of solid phase chemical synthesis of polyproteins are well known in the art and may be found in general texts in the area. *See, e.g.*, H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54-92. For example, proteins may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A protein synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems.

The proteins of the present invention can also be produced by recombinant DNA methods using a cloned or other hLTBP-3 nucleic acid template. Recombinant methods are preferred if a high yield is desired. Expression of an hLTBP-3 gene can be carried out in a variety of suitable host cells, well known to those skilled in the art. For example, the hLTBP-3 gene or fragment thereof (e.g. SEQ ID NOS: 1 or 3) is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned gene is within the scope of the present invention, it is preferred that the gene or fragment thereof be cloned into a suitable extra-chromosomally maintained expression vector so that the coding region of the hLTBP-3 gene is operably-linked to a constitutive or inducible promoter.

The basic steps in the recombinant production of an hLTBP-3 polypeptide are:

a) constructing a natural, synthetic or semi-synthetic DNA encoding an hLTBP-3 polypeptide;

b) integrating said DNA into an expression vector in a manner suitable for expressing the hLTBP-3 polypeptide, either alone or as a fusion protein;

c) transforming or otherwise introducing said vector into an appropriate eucaryotic or prokaryotic cells forming a recombinant host cell;

d) culturing said recombinant host cell in a manner to express the hLTBP-3 polypeptide; and

e) recovering and substantially purifying the hLTBP-3 polypeptide by any suitable means, well known to those skilled in the art.

Expressing Recombinant hLTBP-3 polypeptide in Procaryotic and Eucaryotic Host Cells

Procaryotes may be employed in the production of recombinant hLTBP-3 polypeptide. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31846) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of *E. coli*, bacilli such as *Bacillus subtilis*, enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescens*, various *Pseudomonas* species and other bacteria, such as *Streptomyces*, may also be employed as host cells in the cloning and expression of the recombinant proteins of this invention.

Promoter sequences suitable for driving the expression of genes in procaryotes include β-lactamase [*e.g.* vector pGX2907, ATCC 39344, contains a replicon and β-lactamase gene], lactose systems [Chang et al., *Nature* (London), 275:615 (1978); Goeddel et al., *Nature* (London), 281:544 (1979)], alkaline phosphatase, and the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695)], which is designed to facilitate expression of an open reading frame as a trpE fusion protein under the control of the trp promoter. Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other bacterial promoters, whose nucleotide

sequences are generally known, may be ligated to DNA encoding the protein of the instant invention, using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably-linked to the DNA encoding the desired polyproteins. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or proteins, for example, a glutathione-S-transferase (GST)-hLTBP-3 fusion protein, essentially as described in Smith & Johnson, *Gene*, 67, 31, 1988, herein incorporated by reference. Fusion partners can be removed by enzymatic or chemical cleavage. It is often observed in the production of certain proteins in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired protein, or provides a convenient means of purifying the protein. This is particularly relevant when expressing mammalian proteins in procaryotic hosts. A variety of peptidases (e.g. enterokinase and thrombin) which cleave a polyprotein at specific sites or digest the proteins from the amino or carboxy termini (*e.g.* diaminopeptidase) of the protein chain are known. Furthermore, particular chemicals (*e.g.* cyanogen bromide) will cleave a polyprotein chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. *See e.g.*, P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13, in Protein Purification: From Molecular Mechanisms to Large Scale Processes, American Chemical Society, Washington, D.C. (1990).

In addition to procaryotes, a variety of amphibian expression systems such as frog oocytes, and mammalian cell

systems can be used. The choice of a particular host cell depends to some extent on the particular expression vector used. Exemplary mammalian host cells suitable for use in the present invention include HepG-2 (ATCC HB 8065), CV-1 (ATCC CCL 70), LC-MK_2 (ATCC CCL 7.1), 3T3 (ATCC CCL 92), CHO-K1 (ATCC CCL 61), HeLa (ATCC CCL 2), RPMI8226 (ATCC CCL 155), H4IIEC3 (ATCC CCL 1600), C127I (ATCC CCL 1616), HS-Sultan (ATCC CCL 1884), and BHK-21 (ATCC CCL 10), for example.

A wide variety of vectors are suitable for transforming mammalian host cells. For example, the pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome required for transcription and polyadenylation. A large number of plasmid pSV2-type vectors have been constructed, such as pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-β-globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are widely available from sources such as the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, or the Northern Regional Research Laboratory (NRRL), 1815 N. University Street, Peoria, Illinois, 61604.

Promoters suitable for expression in mammalian cells include the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the glucocorticoid-inducible tyrosine aminotransferase gene, the thymidine kinase gene promoter, and the promoters of the major early and late adenovirus genes.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. German *et al., Porch. Nat. Acad. SCI. (USA)*, 79, 6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and

other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the present invention. This promoter is present in the plasmid pdBPV-MMTneo (ATCC 37224) which can serve as the starting material for the construction of other plasmids of the present invention.

Transfection of mammalian cells with vectors can be performed by a plurality of well known processes including, but not limited to, protoplast fusion, calcium phosphate co-precipitation, electroporation and the like. *See, e.g.*, Maniatis *et al., supra*.

Some viruses also make appropriate vectors. Examples include the adenoviruses, the adeno-associated viruses, the vaccinia virus, the herpes viruses, the baculoviruses, and the rous sarcoma virus, as described in U.S. Patent 4,775,624, incorporated herein by reference.

Eucaryotic microorganisms such as yeast and other fungi are also suitable host cells. The yeast *Saccharomyces cerevisiae* is the preferred eucaryotic microorganism. Other yeasts such as *Kluyveromyces lactis* and *Pichia pastoris* are also suitable. For expression in *Saccharomyces*, the plasmid YRp7 (ATCC-40053), for example, may be used. *See, e.g.*, L. Stinchcomb *et al.*, *Nature*, 282, 39 (1979); J. Kingsman *et al.*, *Gene*, 7, 181 (1979); S. Tschemper *et al.*, *Gene*, 10, 157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp1 auxotrophic mutant.

Purification of Recombinantly-Produced hLTBP-3 polypeptides

An expression vector carrying a cloned hLTBP-3 gene or fragment thereof (e.g. SEQ ID NO: 1 or 3) is transformed or transfected into a suitable host cell using standard methods. Cells that contain the vector are propagated under conditions suitable for expression of a recombinant hLTBP-3 polypeptide. For example, if the recombinant gene or fragment thereof has been placed under the control of an inducible promoter, suitable growth conditions would incorporate the appropriate inducer. The recombinantly-

produced protein may be purified from cellular extracts of transformed cells by any suitable means.

In a suitable process for protein purification, an hLTBP-3 gene or fragment thereof is modified at the 5' end to incorporate several histidine codons. This modification produces an "histidine tag" at the amino terminus of the encoded protein, that enables a single-step protein purification method [i.e. "immobilized metal ion affinity chromatography" (IMAC)], essentially as described in U.S. Patent 4,569,794, which hereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure recombinant hLTBP-3 polypeptide starting from a crude extract of cells that express a modified recombinant protein, as described above.

Production of Antibodies

The proteins of this invention and fragments thereof may be used in the production of antibodies. The instant invention also encompasses single-chain polyprotein binding molecules.

The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the art. *See, e.g.*, C. Milstein, Handbook of Experimental Immunology, (Blackwell Scientific Pub., 1986); J. Goding, Monoclonal Antibodies: Principles and Practice, (Academic Press, 1983). For the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting in a hybridoma that reproduces *in vitro*. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. Each antibody obtained in this way is the clonal product of a single B cell.

Chimeric antibodies are described in U.S. Patent No. 4,816,567, the entire contents of which is herein incorporated by reference. This reference discloses methods

and vectors for the preparation of chimeric antibodies. An alternative approach is provided in U.S. Patent No. 4,816,397, the entire contents of which is herein incorporated by reference. This patent teaches co-expression of the heavy and light chains of an antibody in the same host cell.

The approach of U.S. Patent 4,816,397 has been further refined in European Patent Publication No. 0 239 400. The teachings of this European patent publication are a preferred format for genetic engineering of monoclonal antibodies. In this technology the complementarity determining regions (CDRs) of a human antibody are replaced with the CDRs of a murine monoclonal antibody, thereby converting the specificity of the human antibody to the specificity of a murine antibody.

Single chain antibodies and libraries thereof are yet another variety of genetically engineered antibody technology that is well known in the art. (*See, e.g.* R.E. Bird, *et al., Science* 242:423-426 (1988); PCT Publication Nos. WO 88/01649, WO 90/18430, and WO 91/10737. Single chain antibody technology involves covalently joining the binding regions of heavy and light chains to generate a single polyprotein chain. The binding specificity of the intact antibody molecule is thereby reproduced on a single polyprotein chain.

The antibodies contemplated by this invention are useful in diagnostics, therapeutics or in combinations thereof.

The proteins of this invention or suitable fragments thereof can be used to generate polyclonal or monoclonal antibodies, and various inter-species hybrids, or humanized antibodies, or antibody fragments, or single-chain antibodies. The techniques for producing antibodies are well known to skilled artisans. (*See e.g.* A.M. Campbell, Monoclonal Antibody Technology: Laboratory Techniques in Biochemsitry and Molecular Biology, Elsevier Science Publishers, Amsterdam (1984); Kohler and Milstein, *Nature*

256, 495-497 (1975); Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995.

A protein used as an immunogen may be modified or administered in an adjuvant, by subcutaneous or intraperitoneal injection into, for example, a mouse or a rabbit. For the production of monoclonal antibodies, spleen cells from immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag18 cells, and allowed to become monoclonal antibody producing hybridoma cells in the manner known to the skilled artisan. Hybridomas that secrete a desired antibody molecule can be screened by a variety of well known methods, for example ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al. Exp. Cell Res.* 175, 109-124 (1988); Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995).

For some applications labeled antibodies are desirable. Procedures for labeling antibody molecules are widely known, including for example, the use of radioisotopes, affinity labels, such as biotin or avidin, enzymatic labels, for example horseradish peroxidase, and fluorescent labels, such as FITC or rhodamine (*See e.g.* Enzyme-Mediated Immunoassay, Ed. T. Ngo, H. Lenhoff, Plenum Press 1985; Principles of Immunology and Immunodiagnostics, R.M. Aloisi, Lea & Febiger, 1988).

Labeled antibodies are useful for a variety of diagnostic applications. In one embodiment the present invention relates to the use of labeled antibodies to detect the presence of hLTBP-3 polypeptides. Alternatively, the antibodies could be used in a screen to identify potential modulators of hLTBP-3. For example, in a competitive displacement assay, the antibody or compound to be tested is labeled by any suitable method. Competitive displacement of an antibody from an antibody-antigen complex by a test compound such that a test compound-antigen complex is formed provides a method for identifying compounds that bind hLTBP-3.

Other embodiments of the present invention comprise isolated nucleic acid sequences that encode the proteins of the invention, for example, SEQ ID NO: 1. Also contemplated are related nucleic acids that hybridize to SEQ ID NO: 1, SEQ ID NO: 2, or related fragments thereof, e.g. under high stringency conditions. Such sequences may come, for example, from paralogous or orthologous genes.

The nucleic acids of the invention (e.g. SEQ ID NOS: 1 and 3) and related nucleic acid molecules may be produced by chemical synthetic methods. The synthesis of nucleic acids is well known in the art. *See, e.g.*, E.L. Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, *Methods in Enzymology*, 68:109-151 (1979). Nucleic acids of this invention, including those disclosed herein, could be generated using a conventional DNA synthesizing apparatus, such as the Applied Biosystems Model 380A or 380B DNA synthesizers (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) using phosphoramidite chemistry, thereafter ligating the fragments so as to reconstitute the entire gene. Alternatively, phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. (*See, e.g.*, M.J. Gait, ed., Oligonucleotide Synthesis, A Practical Approach, (1984)).

In an alternative methodology, namely PCR, the DNA sequences disclosed and described herein, can be produced from a plurality of starting materials. For example, starting with a cDNA preparation (e.g. cDNA library) derived from a tissue that expresses an hLTBP-3 gene, suitable oligonucleotide primers complementary to, for example, SEQ ID NO: 1, SEQ ID NO: 3, or to a sub-region therein, for example, are prepared as described in U.S. Patent No. 4,889,818, hereby incorporated by reference. Other suitable protocols for the PCR are disclosed in PCR Protocols: A Guide to Method and Applications, Ed. Michael A. Innis et al., Academic Press, Inc. (1990). Using PCR, any region of

the hLTBP-3 gene(s) can be targeted for amplification such that full or partial length gene sequences may be produced.

The ribonucleic acids of the present invention may be prepared using polynucleotide synthetic methods discussed *supra*, or they may be prepared enzymatically, for example, using RNA polymerase to transcribe a hLTBP-3 DNA template.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. *See*, Maniatis *et al.*, *supra*.

This invention also provides nucleic acids, RNA or DNA, that are complementary to the hLTBP-3 polynucleotides as shown in SEQ ID NOS: 1 or 3 as well as fragments thereof.

Nucleic Acid Probes

The present invention also provides probes and primers useful, for example, in hybridization screens of genomic, subgenomic, or cDNA libraries, as well as hybridization against nucleic acids derived from cell lines or tissues. Such hybridization screens are useful as methods to identify and isolate full length, or identical, homologous or functionally related sequences from human or other organisms.

In one embodiment the present invention relates to the use of a nucleic acid disclosed herein as a probe to identify and isolate full-length genes comprising said nucleic acids. A nucleic acid compound comprising SEQ ID NO: 1, SEQ ID NO: 3, a complementary sequence thereof, or a fragment thereof, which is at least 14 base pairs in length, and which will selectively hybridize to DNA or mRNA encoding hLTBP-3 polypeptide or fragment thereof, or a functionally related protein, is provided. Preferably, the 14 or more base pair compound is DNA. *See e.g.* B. Wallace and G. Miyada, "Oligonucleotide Probes for the Screening of

Recombinant DNA Libraries," In *Meth. Enzym.*, 152, 432-442, Academic Press (1987).

Probes and primers can be prepared by enzymatic or recombinant methods, well known to those skilled in the art (*See e.g.* Sambrook et al. *supra*). A probe may be a single stranded nucleic acid sequence which is complementary in some particular degree to a nucleic acid sequence sought to be detected ("target sequence"). A probe may be labeled with a detectable moiety such as a radio-isotope, antigen, or chemiluminescent moiety. A description of the use of nucleic acid hybridization as a procedure for the detection of particular nucleic acid sequences is described in U.S. Patent No. 4,851,330 to Kohne, entitled "Method for Detection, Identification and Quantitation of Non-Viral Organisms."

Having the DNA sequence of the present invention allows preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to nucleic acids that are homologous or identical to sequences disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding a hLTBP-3 gene or related sequence lends particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of a gene or polynucleotide that encodes an hLTBP-3 polyprotein, using PCR technology.

Preferred nucleic acid sequences employed for hybridization studies, or assays, include probe molecules that are complementary to at least an about

18 to an about 70-nucleotide long stretch of a polynucleotide that encodes an hLTBP-3 polyprotein. Molecules having complementary sequences over stretches greater than 18 bases in length are generally preferred, in order to increase stability and selectivity of the hybrid. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 25 to 40 nucleotides, 55 to 70 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR TM technology of U.S. Pat. No. 4,603,102, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction enzyme sites.

The following guidelines are useful for designing probes with desirable characteristics. The extent and specificity of hybridization reactions are affected by a number of factors that determine the sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The affect of various experimental parameters and conditions are well known to those skilled in the art.

First, the stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing a probe with an appropriate Tm (i.e. melting temperature). The melting profile, including the Tm of a hybrid comprising an oligonucleotide and target sequence, may be determined using a Hybridization Protection Assay. The probe should be chosen so that the length and % GC content result in a Tm about 2°-10° C higher than the temperature at which the final assay will be performed. The base composition of the probe is also a significant factor because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs. Thus, hybridization involving

complementary nucleic acids of higher G-C content will be more stable at higher temperatures.

The ionic strength and incubation temperature under which a probe will be used should also be taken into account. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of molecular hybrids will increase with increasing ionic strength. On the other hand, chemical reagents such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, increase the stringency of hybridization. Destabilization of hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5° C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another even though the one sequence differs merely by a single base. Finally, there can be intramolecular and intermolecular hybrids formed within a probe if there is sufficient self-complementarity. Such structures can be avoided through careful probe design. Computer programs are available to search for this type of interaction.

A probe molecule may be used for hybridizing to a sample suspected of possessing an hLTBP-3 or related nucleotide sequence. The hybridization reaction is carried out under suitable conditions of stringency.

Alternatively, such DNA molecules may be used in a number of techniques including their use as: (1) diagnostic

tools to detect polymorphisms in DNA samples from a human or other mammal; (2) means for detecting and isolating homologs of hLTBP-3 and related polyproteins from a DNA library potentially containing such sequences; (3) primers for hybridizing to related sequences for the purpose of amplifying those sequences; and (4) primers for altering the native hLTBP-3 DNA sequences; as well as other techniques which rely on the similarity of the DNA sequences to those of the hLTBP-3 DNA segments herein disclosed.

Once synthesized, oligonucleotide probes may be labeled by any of several well known methods. *See e.g.* Maniatis *et.al.*, Molecular Cloning (2d ed. 1989). Useful labels include radioisotopes, as well as non-radioactive reporting groups. Isotopic labels include H^3, S^{35}, P^{32}, I^{125}, Cobalt, and C^{18}. Most methods of isotopic labeling involve the use of enzymes and include methods such as nick-translation, end-labeling, second strand synthesis, and reverse transcription. When using radio-labeled probes, hybridization can be detected by autoradiography, scintillation counting, or gamma counting. The detection method selected will depend upon the hybridization conditions and the particular radio isotope used for labeling.

Non-isotopic materials can also be used for labeling, and may be introduced internally into the sequence or at the end of the sequence. Modified nucleotides may be incorporated enzymatically or chemically, and chemical modifications of the probe may be performed during or after synthesis of the probe, for example, by the use of non-nucleotide linker groups. Non-isotopic labels include fluorescent molecules, chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands. In a preferred embodiment of the invention, the length of an oligonucleotide probe is greater than or equal to about 18 nucleotides and less than or equal to about 50 nucleotides. Labeling of an oligonucleotide of the present invention may

be performed enzymatically using [^{32}P]-labeled ATP and the enzyme T4 polynucleotide kinase.

Vectors

Another aspect of the present invention relates to recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. The preferred nucleic acid vectors are those which comprise DNA, for example, SEQ ID NOS: 1 or 3 or a subregion therein.

The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends upon a number of factors including the availability of restriction enzyme sites, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and another), and the number of copies of the gene desired in the host cell.

Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. Regarding promoter sequences, inducible promoters are preferred because they enable high level, regulatable expression of an operably-linked gene. The skilled artisan will recognize a number of suitable promoters that respond to a variety of inducers, for example, carbon source, metal ions, and heat. Other

relevant considerations regarding an expression vector include whether to include sequences for directing the localization of a recombinant protein. For example, a sequence encoding a signal protein (e.g., SEQ ID NO: 5) preceding the coding region of a gene is useful for directing the extra-cellular export of a resulting polyprotein.

The present invention also provides a method for constructing a recombinant host cell capable of expressing the proteins of the invention, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence that encodes a protein of this invention. A suitable host cell is any eucaryotic cell that can accomodate high level expression of an exogenously introduced gene or protein, and that will incorporate said protein into its membrane structure. Vectors for expression are those which comprise an hLTBP-3 polynucleotide as shown in SEQ ID NOS: 1 or 3, as well as any suitable subregion therein. Transformed host cells may be cultured under conditions well known to skilled artisans such that a protein of the present invention is expressed, thereby producing a recombinant hLTBP-3 polypeptide in the recombinant host cell.

For the purpose of identifying compounds having utility as modifiers, agonists, and/or antagonists of cell proliferation, it would be desirable to identify compounds that bind an hLTBP-3 polypeptide of the present invention, and/or modify its activity. A method for determining agents that bind the hLTBP-3 polypeptide and/or activate or inhibit its activity, comprises contacting the hLTBP-3 polypeptide with a test compound and monitoring binding by any suitable means.

The instant invention provides a screening system for discovering compounds that bind the hLTBP-3 polypeptide, said screening system comprising the steps of:

a) preparing an hLTBP-3 polypeptide by any means known to one skilled in the art;

b) exposing said hLTBP-3 polypeptide to a test compound;

c) quantifying the binding of said compound to hLTBP-3 polypeptide by any suitable means, for example, by monitoring the spectroscopic changes in intrinsic tryptophan fluorescence of hLTBP-3 induced by the binding of the compound (Winzor, D.J and Sawyer, W.H., Quantitative Characterization of Ligand Binding. Wiley-Liss, NY. 1995).

Utilization of the screening system described above provides a means to identify compounds that may alter, augment, or inhibit the biological function of hLTBP-3 polypeptides. This screening method may be adapted to large-scale, automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system, allowing for efficient high-volume screening for potential therapeutic agents.

In one embodiment of this aspect of the invention, hLTBP-3 is prepared as described herein, preferably using recombinant DNA technology. A test compound is introduced into a reaction vessel containing an hLTBP-3 polypeptide or fragment thereof. Binding of hLTBP-3 by a test compound is determined by any suitable means. For example, in one method radioactively- labeled or chemically-labeled test compound may be used. Binding of the protein by the test compound is assessed, for example, by quantifying bound label versus unbound label using any suitable method.

Binding of a test compound may also be carried out by a method disclosed in U.S. Patent 5,585,277, which hereby is incorporated by reference. In this method, binding of a test compound to a protein is assessed by monitoring the ratio of folded protein to unfolded protein, for example, by

monitoring sensitivity of said protein to a protease, or amenability for binding of said protein by a specific antibody against the folded state of the protein.

The foregoing screening methods are useful for identifying an agonist and/or antagonist of a hLTBP-3 polypeptide as a lead to a pharmaceutical compound for treating cancer. For example, a compound that binds hLTBP-3, or related fragment thereof, is identified by combining a test compound with hLTBP-3 under conditions that cause the protein to exist in a ratio of folded to unfolded states. If a test compound binds the folded state of the protein, the relative amount of folded protein will be higher than in the case of a test compound that does not bind the protein. The ratio of protein in the folded versus unfolded state is easily determinable by, for example, susceptibility to digestion by a protease, or binding to a specific antibody, or binding to chaperonin protein, or binding to any suitable surface.

In addition, use of antagonists of TGFβ may form the basis of important novel approaches for the treatment of a large spectrum of serious chronic conditions in which excessive TGFβ action appears to be responsible for tissue damage caused by scarring (Border and Noble, 1994, N. Engl. J. Med. 331: 1286-1292; Border and Ruoslahti, 1992, J. Clin. Invest. 90: 1-7). Indeed, in previous studies, antiserum to TGF-β have been shown to protect against scarring in experimental skin lesions and kidney disease (Shah et al., 1992, Lancet, 339: 213-214; Border et al., 1992, Kidney Int. 41: 566-570). Thus, hLTBP-3 can be used as a potent and specific inhibitor of TGF-β for the prevention of fibrotic conditions.

hLTBP-3 polypeptide Therapeutic Applications

In one embodiment, the present invention relates to therapeutic applications in which the inhibition of cell proliferation is therapeutically desirable. For example,

hLTBP-3 is administered to prevent or inhibit tumor growth, or as a treatment for cancers, for example, brain, kidney, bone, or spleenal cancers.

For therapeutic use in preventing or inhibiting tumor growth, or in treating cancer, an effective amount of hLTBP-3 polypeptide is administered to an organism in need thereof in a dose between about 0.1 and 1000 µg/kg body weight. In practicing the methods contemplated, hLTBP-3 can be administered in a single daily dose or in multiple doses per day. The amount per administration will be determined by the physician and depend on such factors as the nature and severity of the disease, and the age and general health of the patient.

The present invention also provides a pharmaceutical composition comprising as the active agent a hLTBP-3 polypeptide or fragment thereof, or a pharmaceutically acceptable non-toxic salt thereof, and a pharmaceutically acceptable solid or liquid carrier. For example, compounds comprising hLTBP-3 can be admixed with conventional pharmaceutical carriers and excipients, and used in the form of tablets, capsules, elixirs, suspensions, syrups, wafers, and the like. The compositions comprising hLTBP-3 will contain from about 0.1% to 90% by weight of the active compound, and more generally from about 10% to 30%. The compositions may contain common carriers and excipients such as corn starch or gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride, and alginic acid. The compounds can be formulated for oral or parenteral administration.

For intravenous (IV) use, the hLTBP-3 polypeptide is administered in commonly used intravenous fluid(s) and administered by infusion. Such fluids, for example, physiological saline, Ringer's solution or 5% dextrose solution can be used.

For intramuscular preparations, a sterile formulation, preferably a suitable soluble salt form of the hLTBP-3

polypeptide (e.g., SEQ ID NOS: 2, 4, or 6) such as the hydrochloride salt, can be dissolved and administered in a pharmaceutical diluent such as pyrogen-free water (distilled), physiological saline or 5% glucose solution. A suitable insoluble form of the compound may be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, e.g. an ester of a long chain fatty acid such as ethyl oleate.

Skilled artisans will recognize that IC_{50} values are dependent on the selectivity of the compound tested. For example, a compound with an IC_{50} which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for a particular target, may be an even better candidate. The skilled artisan will recognize that any information regarding the binding potential, inhibitory activity, or selectivity of a particular compound is useful toward the development of pharmaceutical products.

The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described are merely illustrative and are not intended to limit the present invention in any manner.

EXAMPLE 1

RT-PCR Amplification of hLTBP-3 from mRNA

The hLTBP-3 gene is isolated by reverse transcriptase PCR (RT-PCR) using conventional methods. Total RNA is prepared from a tissue that expresses the hLTBP-3 gene, for example, cartilage, bone, or spleen, using standard methods. First strand cDNA synthesis is achieved using a commercially available kit (SuperScript™ System; Life Technologies) by PCR in conjunction with appropriate primers directed at any suitable region of SEQ ID NO: 1, e.g. the 5′ and 3′ ends of the gene, specifically at nucleotide residues 1 (5′ end) and 3798 (3′ end).

Amplification is carried out by adding to the first strand cDNA (dried under vacuum): 8 µl of 10X synthesis buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl, 25 mM $MgCl_2$, 1 µg/µl BSA); 68 µl distilled water; 1 µl each of a 10 µM solution of each primer; and 1 µl Taq DNA polymerase (2 to 5 U/µl). The reaction is heated at 94° C for 5 min. to denature the RNA/cDNA hybrid. Then, 15 to 30 cycles of PCR amplification are performed using any suitable thermal cycle apparatus. The amplified sample may be analyzed by agarose gel electrophoresis to check for an appropriately-sized fragment.

EXAMPLE 2

Production of a Vector for Expressing hLTBP-3 in a Host Cell

An expression vector suitable for expressing hLTBP-3 or fragment thereof in a variety of procaryotic host cells, such as *E. coli* is easily made. The vector contains an origin of replication (Ori), an ampicillin resistance gene (Amp) useful for selecting cells which have incorporated the vector following a tranformation procedure, and further comprises the T7 promoter and T7 terminator sequences in operable linkage to a hLTBP-3 coding region. Plasmid pET11A (obtained from Novogen, Madison WI) is a suitable parent plasmid. pET11A is linearized by restriction with endonucleases NdeI and BamHI. Linearized pET11A is ligated to a DNA fragment bearing NdeI and BamHI sticky ends and comprising all or part of the coding region of the hLTBP-3 gene as disclosed by, for example, SEQ ID NO: 1.

The hLTBP-3 gene used in this construction may be slightly modified at the 5' end (amino terminus of encoded protein) in order to simplify purification of the encoded protein product. For this purpose, an oligonucleotide encoding 8 histidine residues is inserted at or near the 5' end of the hLTBP-3 gene, so as to maintain the correct reading frame. Placement of the histidine residues at the amino terminus of the encoded protein serves to enable the IMAC one-step protein purification procedure.

EXAMPLE 3

Recombinant Expression and Purification of hLTBP-3 polypeptide

An expression vector that carries a gene encoding hLTBP-3 or fragment thereof and which gene is operably-linked to an expression promoter is transformed into *E. coli* BL21 (DE3)(*hsd*S *gal* λcIts857 *ind*1*Sam*7*nin*5*lac*UV5-T7gene 1) using standard methods. Transformants, selected for resistance to ampicillin, are chosen at random and tested for the presence of the vector by agarose gel electrophoresis using quick plasmid preparations. Colonies which contain the vector are grown in L broth and the protein product encoded by the vector-borne gene is purified by immobilized metal ion affinity chromatography (IMAC), essentially as described in US Patent 4,569,794.

Briefly, the IMAC column is prepared as follows. A metal-free chelating resin (e.g. Sepharose 6B IDA, Pharmacia) is washed in distilled water to remove preservative substances and infused with a suitable metal ion [e.g. Ni(II), Co(II), or Cu(II)] by adding a 50mM metal chloride or metal sulfate aqueous solution until about 75% of the interstitial spaces of the resin are saturated with colored metal ion. The column is then ready to receive a crude cellular extract containing the recombinant protein product.

After removing unbound proteins and other materials by washing the column with any suitable buffer, pH 7.5, the bound protein is eluted in any suitable buffer at pH 4.3, or preferably with an imidizole-containing buffer at pH 7.5.

EXAMPLE 4

Tissue Distributuion of hLTBP-3 mRNA

hLTBP-3 mRNA is detected in a mammalian tissue by Northern analysis. Total RNA from different tissues or cultured cells is isolated by a standard guanidine

chloride/phenol extraction method, and poly-A$^+$ RNA is isolated using oligo(dT)-cellulose type 7 (Pharmacia). Electrophoresis of RNA samples is carried out in formaldehyde followed by capillary transfer to Zeta-Probe TM nylon membranes (Bio-Rad, Hercules, Calif.). SEQ ID NO: 1 or SEQ ID NO: 3 is used as a template for generating probes using a MultiPrime™ random priming kit (Amersham, Arlington Heights, Ill.). The efficiency of the labeling reaction is approximately 4 x 10^{10} cpm incorporated per µg of template. The hybridization buffer contains 0.5M sodium phosphate, 7% SDS (wt/vol), 1% BSA (wt/vol), and 1 mM EDTA. Prehybridization is carried out in hybridization buffer at 65° C for 2 h, and ^{32}P-labeled probe is added and incubation continued overnight. Filters are washed in Buffer A (40 mM sodium phosphate pH 7.2, 5% SDS [wt/vol], 0.5% BSA [wt/vol], and 1 mM EDTA) at 65° C for 1 h, then in Buffer B (40 mM sodium phosphate, pH 7.2, 1% SDS [wt/vol], and 1 mM EDTA) at 65° C for 20 min. Filters are then air-dried and exposed to Kodak X-OMAT AR film at -80° C with an intensifying screen.

EXAMPLE 5

Detecting Ligands that Bind hLTBP-3 Using a Protein-Interaction Assay

The wells of an ELISA plate are coated with chaperonin or other proteins known to interact with hLTBP-3 by incubation for several hours with a 4 µg/ml solution of the protein in Tris-buffered Saline (TBS: 10 mM Tris-HCl, pH7.5, 0.2M NaCl). Plates are washed 3 times with TBS containing 0.1% Tween-20 (TBST). Then, a mixture of hLTBP-3 polypeptide (sufficient amount to saturate about 50% of the binding sites) and test compound (10^{-9} to 10^{-5} M) in about 50 µl volume is added to each well of the plate for an incubation of about 60 minutes. Aliquots from each well are then transferred to the wells of fresh plates and incubation is continued for 60 minutes at room temperature, followed by 3 washes with TBST. Next, about 50 µl of an antibody specific

for hLTBP-3 in 5% nonfat dry milk is added to each well for 30 minutes at room temperature. After washing, about 50 µl of goat anti-rabbit IgG alkaline phosphatase conjugate at an appropriate dilution in TBST plus 5% nonfat dry milk is added to each will and incubated 30 minutes at room temperature. The plates are washed again with TBST, and 0.1 ml of 1 mg/ml p-nitrophenylphosphate in 0.1% diethanolamine is added. Color development (proportional to bound alkaline phosphatase antibody conjugate) is monitored with an ELISA plate reader. When binding by the test ligand has occurred, ELISA analysis reveals hLTBP-3 in solution at higher concentrations than in the absence of test ligand.

EXAMPLE 6

Production of an Antibody to hLTBP-3 polypeptide

Substantially pure hLTBP-3 polypeptide or fragment thereof is isolated from transfected or transformed cells using any of the well known methods in the art, or by a method specifically disclosed herein. Concentration of protein is adjusted, for example, by filtration through an Amicon filter device such that the level is about 1 to 5 µg/ml. Monoclonal or polyclonal antibody is prepared as follows.

Monoclonal antibody can be prepared from murine hybridomas according to the method of Kohler and Milstein (*Nature*, 256, 495, 1975), or a modified method thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the hLTBP-3 polypeptide (e.g. SEQ ID NOS:2, 4, or 6), fragment thereof, or fusion protein thereof, over a period of a few weeks. The mouse is then sacrificed, and antibody producing cells of the spleen are isolated. Spleen cells are fused with mouse myeloma cells using polyethylene glycol. Fused cells that produce antibody are identified by any suitable immunoassay, for example, ELISA, as described in E. Engvall, *Meth. Enzymol.*, 70, 419, 1980.

Polyclonal antiserum can be prepared by well known methods (*See e.g.* J. Vaitukaitis *et.al. Clin. Endocirnol. Metab.* 33, 988, 1971) that involve immunizing suitable animals with a hLTBP-3 polypeptide, fragments thereof, or fusion proteins thereof. Small doses (e.g. nanogram amounts) of antigen administered at multiple intradermal sites are the most reliable method.

EXAMPLE 7

Cell Proliferation Assays for hLTBP-3 Activity

The effect of hLTBP-3 on reversing inhibition of cell growth induced by TGFβ can be assessed in various cell lines including, but not limited to, the Mv1Lu cell line (Bottinger et al., 1996, Proc. Natl. Acad. Sci. USA 93: 5877-5882). Briefly, MV1Lu cells (1 x 10^6 cells/well in 24 well dishes) are grown in the presence of TGF-β (1-10 pM) and varying concentrations of recombinant hLTBP-3. Cell growth is monitored by [^{3}H]thymidine incorporation into cellular DNA over a 24 hour time period. Radioactivity can be measured by the scintillation proximity technique in a Wallac 1450 microbeta counter or by traditional scintillation counting.

EXAMPLE 8

Cell Based Assays for hLTBP-3 Activity

Transfected cell lines including, but not limited to, MV1Lu can also be employed to monitor the modulation of TGF-β activated gene expression by exogenously applied hLTBP-3. For instance, the induction of the plasminogen activator inhibitor 1 (PAI-1) promoter by TGF-β can be monitored in cells stably transfected with the PAI-1 promoter fused to a reporter gene (i.e., luciferase) (Abe, et al., 1994, Anal. Biochem. 216: 276-284). Stable cell lines would be incubated for 48 hours with TGF-β and varying concentration

of hLTBP-3 polypeptide. The amount of luciferase protein produced would be quantitated directly by the photons emitted by cell lysates incubated with luciferin substrate using a luminometer.

To elucidate its role on endothelial cell anticoagulant and profibrinolytic activities, the effects of hLTBP-3 over-expression on two well-characterized markers of endothelial cell function, thrombomodulin (TM) and plasminogen activator inhibitor-1 (PAI-1), can be examined. Both markers are known to be responsive to TGFβ, with TM being suppressed and PAI-1 being activated.

These experiments can also be conducted in various cell lines including, but not limited to, SVHA-1 (an SV40 transformed human aortic endothelial cell line) and ECV304 (a spontaneously transformed human umbilical vein endothelial cell line) (ATCC CRL-1998). The cells are maintained in DMEM/F-12 (3:1), a medium comprised of a 3:1 v/v mixture of Dulbecco's Modified Eagle's Medium and Ham's nutrient mixture F-12. The basal medium is supplemented with 10 nM selenium, 50 μM 2-aminoethanol, 20 mM HEPES, 50 μg/ml gentamicin, and 5% fetal bovine serum (FBS).

A. PAI-1 Promoter activity

In order to determine the effects of hLTBP-3 on PAI-1 secretion and promoter activity, the PAI-1 basal promoter driving the expression of the CAT indicator gene (pOCAT2336) and a TGFβ hyper-responsive PAI-1/TRE promoter construct (3TP-lux) driving the expression of the luciferase indicator gene are used. hLTBP-3 expressing vectors are constructed by inserting the hLTBP-3 coding sequence, or fragments thereof, into any suitable expression vector such as pN8e23/3xFlu2 or any other standard expression vectors. ECV304 cells are seeded in 6-well plates to 80% confluence. DNA is transfected at a concentration of 1 μg per well for pOCAT2336 and 3TP-lux

and 5 µg per well for the hLTBP-3 insert containing vector with lipofectin reagent (Gibco/Life Technologies, Gaithersburg, MD). Expressed CAT protein from the PAI-1 basal promoter construct was determined using a CAT ELISA kit (Boehringer Mannheim; Indianapolis, IN). Chemiluminescence resulting from expression of the luciferase gene is determined as a measure of the effect on the PAI-1/TRE promoter. The plates are read kinetically and data expressed in terms of promoter activity relative to control.

These experiments will detect any hLTBP-3 dependent effect on the PAI-1 promoter due to the highly TGFβ-responsive transcriptional element based on the PAI-1 promoter (p3TP-Lux) as well as with the basal PAI-1 promoter (pPAI-CAT). In particular, hLTBP-3 over-expression should result in a greater effect using the more TGFβ-sensitive 3TP-lux plasmid.

In addition to the ability of hLTBP-3 over-expression to regulate the activity of artificial promoter constructs, the actual level of PAI-1 secretion from the cells can also be measured as a marker of hLTBP-3 effects on TGFβ regulated activities.

B. Thrombomodulin activity

Determination of thrombomodulin anticoagulant activity can be performed in confluent cultures of SVHA-1 cells. The cultures are washed once with Hank's Basal Salt Solution to remove serum proteins and incubated with serum-free medium (DMEM/F-12 medium, 20 mM-HEPES, pH 7.5, 50 mg/ml gentamicin, 1 µg/ml human transferrin and 1 µg/ml bovine insulin) containing 400 nM recombinant human proteins (made according to techniques as set forth in U.S. Patent No. 4,981,952) and 10 nM human thrombin (Sigma; St. Louis, MO). Cultures are incubated at 37°C, and at various times medium is removed and added to an equal volume of a solution of 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mg/ml BSA, and 10 U/ml hirudin. The

samples are incubated in the hirudin-containing buffer for 5 minutes to inhibit thrombin activity. In all experiments, samples of the protein C/thrombin solution are incubated in wells without cells to determine basal levels of thrombin-catalyzed activation of protein C.

The amount of activated protein C generated is determined by adding chromogenic substrate (S2366) (Chromogenix, Mölndal, Sweden) to a final concentration of 0.75 mM, and measuring the change in absorbance units/minute at 405 nm in a kinetic micro-titer plate reader. Results are expressed in terms of maximal response to TGFβ.

The amount of activated protein C generated is directly related to the level of surface TM. The effect of hLTBP-3 over-expression on TM activity on the surface of the cells is a reflection of its effect on TGFβ activity.

EXAMPLE 9

EFFECT OF INHIBITING hLTBP-3 WITH ANTISENSE DNA

The oligonucleotides used in antisense experiments are synthesized using phosphorothioates and C-5 propyne pyrimidines. Antisense oligodeoxynucleotides (oligos) are designed to hybridize to the 5′ region of the hLTBP-3 mRNA. Cells are plated in 96-well plates at a density of 2000 or 5000 cells/well and allowed to attach overnight in DMEM/F-12 5% FBS. After washing monolayers with serum free medium (SFM), 1 nmol of each oligo is introduced in 100 µl of SFM. Control wells containing SFM with vehicle alone are included in addition to the sense strand oligo controls. After an overnight incubation in the presence of oligos, test wells are rinsed with SFM and re-charged with oligo overnight as above. On the fourth day each experimental condition is treated with or without TGFβ at a concentration of 1 ng/ml (a concentration found to be optimal for PAI-1 and TM response). PAI-1 levels

are assayed 16 hours later from the culture supernatant using a commercially available PAI-1 ELISA kit (American Diagnostica Inc., Greenwich, CT). Cell surface TM levels are assayed in SVHA-1 cells indirectly by measuring the ability of the cell surface TM to activate human protein C.

After the conditioned medium is removed for the PAI-1 assay it is replaced with 100 µl of SFM containing 25 µg/ml human protein C and 0.5 units/ml thrombin. After 1 hour incubation at room temperature, 75 µl aliquots are removed to 96-well plates, each test well containing 50 µl of 10 U/ml hirudin in activation buffer (20 mM tris pH 7.4, 150 mM NaCl), and incubated 5-10 minutes with agitation. Activated human protein C is then assayed (as a measure of TM activity) by adding 50 µl of the chromogenic substrate S2366 and measuring the change in absorbance at 405 nm on a 5 minute kinetic run.

Antisense oligos to hLTBP-3 can block the TGFβ-dependent suppression of TM surface levels, as measured by the ability of the cells to support the thrombin-dependent activation of human protein C.

EXAMPLE 10

EFFECT OF hLTBP-3 ON TGFβ PRODUCTION

A. TFGβ/CAT Transfection

In order to determine hLTBP-3 effects on TGFβ promoter activity, a TGFβ3 promoter construct driving CAT expression (1 µg) was co-transfected into ECV304 cells with a hLTBP-3 polypeptide encoding pcineo vector (5 µg), a TGFβ polypeptide encoding pcineo vector (5 µg), or both. Controls cells are co-transfected with 1 µg TGFβ3 promoter construct and 10 µg pcineo vector not either TGFβ or hLTBP-3 (Promega, Madison, WI).

Transfection is initiated by plating ECV304 cells at 3 x 10^5 cells per well in a 6-well plate with DMEM/F12 media in 5% fetal bovine serum (FBS). The cells are allowed to attach overnight at 37°C.

Twenty µl lipofectin are diluted with serum-free medium to a total volume of 200 µl per transfection and placed at room temperature for 30 to 60 minutes. Eleven µg DNA is diluted with 200 µl of serum-free medium and mixed with the lipofectin solution. The resulting reagent mixture is incubated at room temperature for 15 minutes.

The medium is aspirated from the culture plates, and the cultures are ished twice with PBS. Two ml of medium are added per well, and the cells are incubated at 37°C 30 minutes prior to adding the lipofectin reagent dropwise to the cultures. The cultures are then incubated 4-6 hours at 37°, the medium is aspirated, and fresh DMEM/F12 5% FBS is added.

After culturing for 24 hours, the cells are washed twice with phosphate buffered saline (PBS), and serum free DMEM medium containing 100 µg/ml Cohn's fractionated bovine serum albumin (BSA) and 2 ng/ml TGFβ3 (R&D Systems) is added. The cells are incubated overnight at 37°C and supernatants are collected and stored frozen. The cells are washed twice with PBS, lysed, and expressed CAT activity is measured kinetically as in Example 1. Lysates are normalized in BCA assay measuring total protein concentration.

B. Endogenous TGFβ

Endogenous levels of TGFβ1 and TGFβ3 secreted into the supernatant of the above cultures can also be evaluated by ELISA. Briefly, 96-well plates are coated with 0.5 µg/ml TGFβ RII receptor (R&D systems) at 100 µl per well in PBS. Plate sealer is added and the plates are stored at 4°C overnight.

The plates are then washed three times with 0.1% Tween 20 in PBS, blocked with 300 µl PBS containing 5% Tween 20 and 5% sucrose for 1-3 hours at room temp.

Latent TGFβ3 is activated by adding 0.1 ml 1N HCL to 0.5 ml supernatant, and incubating 10 minutes at room temperature. The mixture is neutralized with 0.1 ml 1.2 N NaOH and 0.5 M Hepes. The blocking mixture is removed from the prepared plates, and samples are added at 200 µl per well. Standard TGFβ3, serially diluted from 2 ng/ml to 0.016 ng/ml in PBS 3% BSA, is added at 200 µl per well.

The plates are incubated for two hours and washed as before. Rabbit anti-TGFβ3 (Santa Cruz catalog# SC-082) is prepared at 1 µg/ml in PBS 3% BSA, added to the plates at 100 µl per well, incubated for one hour at room temp., and washed as before. Goat anti-Rabbit IgG alkaline phosphate conjugate at 1/250 dilution in PBS 3% goat serum is added to the plates at 100 µl per well, incubated one hour, and washed as before. One PnPP tablet (Sigma) is prepared in 3 ml H_2O, added at 100 µl per well. The plates are incubated for 20-30 minutes and read at OD 405 nm.

Latent TGFβ1 is activated as described for TGFβ3 and assayed according to directions in a commercial TGFβ1 ELISA kit (R&D Systems).

hLTBP-3 expression is expected to inhibit TGF promoters and the secretion of TGFβ.

WE CLAIM:

1. An isolated hLTBP-3 polypeptide comprising at least 20 contiguous amino acids of a polypeptide selected from the group consisting of:

a) SEQ ID NO: 2;

b) SEQ ID NO: 4; and

c) SEQ ID NO: 6.

2. An isolated nucleic acid encoding an hLTBP-3 polypeptide of claim 1.

3. An isolated nucleic acid that encodes at least one hLTBP-3 polypeptide of claim 1 wherein said nucleic acid comprises at least 14 contiguous nucleotides from a polynucleotide selected from the group consisting of:

a) SEQ ID NO: 1; and

b) SEQ ID NO: 3.

4. An isolated nucleic acid molecule comprising a polynucleotide that hybridizes under stringent conditions to at least 14 contiguous nucleotides of SEQ ID NO: 1, SEQ ID NO: 3, or a polynucleotide complementary thereto.

5. An isolated nucleic acid that encodes an hLTBP-3 polypeptide which can antagonize TGFβ activity, wherein said nucleic acid hybridizes under stringent conditions to at least 20 contiguous nucleotides of SEQ ID NOS: 1, 3, or a polynucleotide complimentary thereto.

6. A vector comprising said isolated nucleic acid of any of the claims 2-5.

7. A vector, as in claim 6, wherein said isolated nucleic acid compound is operably-linked to a promoter sequence.

8. A host cell comprising a vector of claim 6 or claim 7.

9. A host cell comprising a vector of claim 6 or claim 7.

10. A method for constructing at least one recombinant host cell, which comprises introducing into said host cell a nucleic acid according to any of claims 2-5.

11. A method for expressing at least one hLTBP-3 polypeptide in a recombinant host cell of claim 10, wherein said method comprises culturing at least one said recombinant host cell under conditions suitable for expression of said hLTBP-3 polypeptide.

12. A method for identifying compounds that bind an isolated hLTBP-3 polypeptide according to claim 1, which comprises:

a) admixing said isolated hLTBP-3 polypeptide with at least one test compound; and

b) detecting at least one binding interaction between said hLTBP-3 polypeptide and at least one said compound.

13. An antibody that selectively binds an epitope specific for an hLTBP-3 polypeptide according to claim 1.

14. A composition comprising an hLTBP-3 polypeptide according to claim 1 and at least one carrier, excipient, or diluent thereof.

15. A method for inhibiting tissue growth *in vitro* or *in vivo*, which comprises administering to a patient in need thereof a tissue growth inhibiting effective amount of at least one composition according to claim 14.

16. A method for inhibiting tumor growth, which comprises administering to a patient in need thereof a tumor cell growth inhibiting effective amount of at least one composition according to claim 14.

17. An antibody that binds at least one epitope specific to a polypeptide comprising at least 20 amino acids of a polypeptide selected from the group consisting of:

a) SEQ ID NO:2;
b) SEQ ID NO:4;
c) SEQ ID NO:5; and
d) SEQ ID NO:6.

18. A method for stimulating tissue growth, which comprises administering to a patient in need thereof a tissue growth stimulating effective amount of at least one composition according to claim 14.

19. A method for inhibiting tissue growth, which comprises administering to a patient in need thereof a tissue growth stimulating effective amount of a compound which binds to an hLTBP-3 polypeptide according to claim 1.

20. A method for inhibiting tumor growth, which comprises administering to a patient in need thereof a tumor growth inhibiting effective amount of a compound which binds to an hLTBP-3 polypeptide according to claim 1.

21. A method for stimulating tissue growth, which comprises administering to a patient in need thereof a tissue stimulating effective amount of a compound which binds to an hLTBP-3 polypeptide according to claim 1.

22. A method for modulating at least one TGFβ regulatable activity in at least one cell, comprising contacting said at least one cell with at least one compound that modulates expression of, or the activity of the protein product of, a nucleic acid molecule having at least 90% identity to at least 40 contiguous nucleotides of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a nucleic acid molecule that is complementary to SEQ ID NO:1, a nucleic acid molecule that is complementary to SEQ ID NO:3, and at least one fragment thereof.

23. The method of claim 22 wherein the nucleic acid molecule has at least 95% identity to at least 40 contiguous nucleotides of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, a nucleic acid molecule that is complementary to SEQ ID NO:1, a nucleic acid molecule that is complementary to SEQ ID NO:3, and at least one fragment thereof.

24. The method of claims 22 and 23 wherein said TGFβ regulatable activity is at least one selected from the group consisting of:

(a) plasminogen activator inhibitor-1 expression;
(b) plasminogen activator inhibitor 1 activity;
(c) plasminogen activator inhibitor-1 secretion;
(d) thrombomodulin expression;
(e) thrombomodulin activity;
(f) TGFβ secretion; and
(g) cellular proliferation.

25. A method for modulating a TGFβ regulatable activity, comprising administering to a cell, cells, or a patient in need of such treatment, a protein encoded by a nucleic acid molecule having at least 90% identity to at least 40 contiugous nucleotides of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and at least one fragment thereof.

26. A method for modulating a TGFβ regulatable activity, comprising administering to at least one cell, an organism, or a patient in need of such treatment, an antisense nucleic acid molecule having a nucleotide sequence complementary to at least 10 contiguous nucleotides of an mRNA transcribed from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and at least one fragment thereof, wherein said antisense nucleic acid molecule hybridizes to said contiguous sequence such that translation of said mRNA is inhibited.

27. A method for the prevention and/or treatment of a disease arising from cellular effects induced by TGFβ, said method comprising administering to a patient in need of such treatment a compound that modulates expression of, or the activity of the protein product of, a nucleic acid molecule comprising at least 40 nucleotides selected from the group consisting of:

a) SEQ ID NO:1;
b) SEQ ID NO:3; and
c) at least one fragment thereof.

28. A method according to claim 27 wherein the disease to be prevented or treated is at least one selected from the group consisting of: cancer, fibrosis, osteoporosis, myocardial infarction, congestive heart failure, dilated

cardiomyopathy, deep venous thrombosis, disseminated intravascular thrombosis, stroke, sepsis, injuries involving major tissue damage and trauma, systemic inflammatory response syndrome, sepsis syndrome, septic shock, multiple organ dysfunction syndrome (including DIC), atherosclerotic plaque rupture, and associated sequela arising therefrom.

29. The use of a compound for the manufacture of a medicament for the treatment or prevention of a disease in which TGFβ is responsible for inducing cellular effects that lead to said disease wherein said compound modulates expression of, or the activity of the protein product of, a nucleic acid molecule having at least 90% homology from a molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and fragments thereof.

30. The use of a compound for the manufacture of a medicament for the prevention of a disease in which TGFβ is responsible for inducing cellular effects that lead to said disease wherein said compound has at least 90% identity to a member selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and fragments thereof.

31. The use of a compound as claimed in Claim 29 or 30 for the manufacture of a medicament for the prevention and/or treatment of disease wherein the disease to be prevented is selected from the group consisting of cancer, fibrosis, osteoporosis, myocardial infarction, congestive heart failure, dilated cardiomyopathy, deep venous thrombosis, disseminated intravascular thrombosis, stroke, sepsis, injuries involving major tissue damage and trauma, systemic inflammatory response syndrome, sepsis syndrome, septic shock, multiple organ dysfunction syndrome (including DIC), atherosclerotic plaque rupture, and associated sequela arising therefrom.

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(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: EDMONDS, Brian, Taylor [US/US]; 12990 Brighton Lane, Carmel, IN 46032 (US).

(74) Agents: WEBSTER, Thomas, D. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).

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(57) Abstract

The invention provides isolated nucleic acid compounds encoding polypeptides and fragments thereof, said polypeptides being related to the human TGFβ latent binding protein (hLTBP) family. This invention also provides vectors and transformed heterologous host cells for expressing said polypeptides, a method of using said nucleic acids and polypeptides for inhibiting TGFβ dependent activities in vitro or in vivo, as well as pharmaceutical compositions comprising said nucleic acids or polypeptides. Also provided are assays used to identify compounds which bind to said polypeptides.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

[x] Declaration Submitted with Initial Filing

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Attorney Docket Number	**X-12239**
First Named Inventor	**Brian Taylor Edmonds**
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As a below named Inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole Inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the Invention entitled:

HUMAN LATENT TRANSFORMING GROWTH FACTOR-β BINDING PROTEIN 3

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II hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

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60/098,766	08/01/1998

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Attorney Name	Reg. No.
Arvie J. Anderson	45,263
Lynn D. Apelgren	45,341
Robert A. Armitage	27,417
Brian P. Barrett	39,597
Michael T. Bates	34,121
Roger S. Benjamin	27,025
William R. Boudreaux	35,796
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Paul R. Cantrell	36,470
Charles E. Cohen	34,565
Robert A. Conrad	32,089
Donald L. Corneglio	30,741
Gregory A. Cox	P47,504
Paul R. Darkes	33,862
Paula K. Davis	P47,517
Elizabeth A. Dawalt	44,646
John C. Demeter	30,167
Manisha A. Desai	43,585
Joanne Longo Feeney	35,134
Paul J. Gaylo	36,808
Francis O. Ginah	44,712
Amy E. Hamilton	33,894
Frederick D. Hunter	26,915
Thomas E. Jackson	33,064
Charles Joyner	30,466
Gerald P. Keleher	43,707
James J. Kelley	41,888

Attorney Name	Reg. No.
Paul C. Kimball	34,641
Paul J. Koivuniemi	31,533
Robert E. Lee	27,919
Kirby Lee	P47,744
James P. Leeds	35,241
Nelsen L. Lentz	38,537
Janet T. McClain	36,863
Douglas K. Norman	33,267
Arleen Palmberg	40,422
Raymond S. Parker, III	34,893
Thomas G. Plant	35,784
Edward Prein	37,212
James J. Sales	33,773
Michael J. Sayles	32,295
Robert L. Sharp	45,609
David M. Stemerick	40,187
Mark J. Stewart	43,936
Robert D. Titus	40,206
Robert C. Tucker	45,165
Tina M. Tucker	47,145
MaCharri Vomdran-Jones	36,711
Gilbert T. Voy	43,972
Andrea C. Walsh	34,988
Thomas D. Webster	39,872
Lawrence T. Welch	29,487
Alexander Wilson	P45,782

☐ Additional registered practitioner(s) named on a supplemental sheet attached hereto.

Direct all correspondence to:

Name	ELI LILLY AND COMPANY						
Address	ATTN: Robert L. Sharp						
Address	LILLY CORPORATE CENTER/DC1104						
City	INDIANAPOLIS	**State**	INDIANA	**ZIP**	46285		
Country		**Telephone**	(317) 276-5332	**Fax**	(317) 276-3861		

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-C

Name of Sole or First Inventor: ☐ **A Petition has been filed for this unsigned inventor**

Given Name	Middle Name	Family Name	Suffix e.g. Jr.
Edmonds	Taylor	Brian	

Inventor's Signature X [signature] **Date** 2/27/01

Residence: City	Carmel	**State**	IN	**Country**	US	**Citizenship**	US
Address	12990 Brighton Lane						
Post Office Address	SAME AS ABOVE						
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☐ **Additional Inventors are being named on supplement sheet(s) attached hereto.**

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<141> Current Filing Date: 2001-06-08

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Asp	Pro	Pro	Gly	Pro	Gly	Glu	Gly	Pro	Pro	Ala	Gln	His	Ala	Ala	Phe
145					150					155					160
Leu	Val	Pro	Leu	Gly	Pro	Gly	Gln	Ile	Ser	Ala	Glu	Gly	Thr	Arg	Gln
				165					170					175	
Leu	Ala	Asn	Pro	Gly	Arg	Ser	Pro	Val	Gly	Gly	His				
			180					185							

<210> 5
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Signal peptide sequence

<400> 5

Met	Arg	Gln	Ala	Gly	Gly	Leu	Gly	Leu	Leu	Ala	Leu	Leu	Leu	Leu	Ala
1				5					10					15	
Leu	Leu	Gly	Pro	Gly	Gly	Arg	Gly	Val	Gly	Arg	Pro	Gly	Ser	Gly	Ala
			20					25					30		

Gln Ala Gly Ala Gly Arg Trp Ala Gln
35 40

<210> 6
<211> 1257
<212> PRT
<213> Homo sapiens

<220>
<223> Xaa = any amino acid

<400> 6
Arg Gly Ala Gly Gly Gly Gly Ala Leu Ala Arg Glu Arg Phe Lys Val
1 5 10 15

Val Phe Ala Pro Val Ile Cys Lys Arg Thr Cys Leu Lys Gly Gln Cys
20 25 30

Arg Asp Ser Cys Gln Gln Gly Ser Asn Met Thr Leu Ile Gly Glu Asn
35 40 45

Gly His Ser Thr Asp Thr Leu Thr Gly Ser Gly Phe Arg Val Val Val
50 55 60

Cys Pro Leu Pro Cys Met Asn Gly Gly Gln Cys Ser Ser Arg Asn Gln
65 70 75 80

Cys Leu Cys Pro Pro Asp Phe Thr Gly Arg Phe Cys Gln Val Pro Ala
85 90 95

Gly Gly Ala Gly Gly Gly Thr Gly Gly Ser Gly Pro Gly Leu Ser Arg
100 105 110

Thr Gly Ala Leu Ser Thr Gly Ala Leu Pro Pro Leu Ala Pro Glu Gly
115 120 125

Asp Ser Val Ala Ser Lys His Ala Ile Tyr Ala Val Gln Val Ile Ala
130 135 140

Asp Pro Pro Gly Pro Gly Glu Gly Pro Pro Ala Gln His Ala Ala Phe
145 150 155 160

Leu Val Pro Leu Gly Pro Gly Gln Ile Ser Ala Glu Gly Thr Arg Gln
165 170 175

Leu Ala Asn Pro Gly Arg Ser Pro Val Gly Gly His Xaa Gly Gly Gln
180 185 190

Gly Arg Ala Gly Ser Ala Leu Glu Glu Leu Ser Ala Val Thr Arg Gly
195 200 205

Ala Val Arg Ala Ala Leu Arg Pro Pro Arg Pro Pro Pro Val Gln Ala
210 215 220

Pro Pro Pro Val Val Asn Val Arg Val His His Pro Pro Glu Ala Ser
225 230 235 240

Val Gln Val His Arg Ile Glu Ser Ser Asn Ala Glu Ser Ala Ala Pro
245 250 255

Ser Gln His Leu Leu Pro His Pro Lys Pro Ser His Pro Arg Pro Pro
260 265 270

Thr Gln Lys Ser Leu Gly Arg Cys Phe Gln Asp Thr Leu Pro Lys Gln
275 280 285

Pro Cys Gly Ser Asn Pro Leu Pro Gly Leu Thr Lys Gln Glu Asp Cys
290 295 300

Cys Gly Ser Ile Gly Thr Ala Trp Gly Gln Ser Lys Cys His Lys Cys
305 310 315 320

Pro Gln Leu Gln Tyr Thr Gly Val Gln Lys Pro Gly Pro Val Arg Gly
325 330 335

Glu Val Gly Ala Asp Cys Pro Gln Gly Tyr Lys Arg Leu Asn Ser Thr
340 345 350

His Cys Gln Asp Ile Asn Glu Cys Ala Met Pro Gly Val Cys Arg His
355 360 365

Gly Asp Cys Leu Asn Asn Pro Gly Ser Tyr Arg Cys Val Cys Pro Pro
370 375 380

Gly His Ser Leu Gly Pro Ser Arg Thr Gln Cys Ile Ala Asp Lys Pro
385 390 395 400

Glu Glu Lys Ser Leu Cys Phe Arg Leu Val Ser Pro Glu His Gln Cys
405 410 415

Gln His Pro Leu Thr Thr Arg Leu Thr Arg Gln Leu Cys Cys Cys Ser
420 425 430

Val Gly Lys Ala Trp Gly Ala Arg Cys Gln Arg Cys Pro Thr Asp Gly
435 440 445

Thr Ala Ala Phe Lys Glu Ile Cys Pro Ala Gly Lys Gly Tyr His Ile
450 455 460

Leu Thr Ser His Gln Thr Leu Thr Ile Gln Gly Glu Ser Asp Phe Ser
465 470 475 480

Leu Phe Leu His Pro Asp Gly Pro Pro Lys Pro Gln Gln Leu Pro Glu
485 490 495

Ser Pro Ser Gln Ala Pro Pro Pro Glu Asp Thr Glu Glu Glu Arg Gly
500 505 510

Val Thr Thr Asp Ser Pro Val Ser Glu Glu Arg Ser Val Gln Gln Ser
515 520 525

His Pro Thr Ala Thr Thr Thr Pro Ala Arg Pro Tyr Pro Glu Leu Ile
530 535 540

Ser Arg Pro Ser Pro Pro Thr Met Arg Trp Phe Leu Pro Asp Leu Pro
545 550 555 560

Pro Ser Arg Ser Ala Val Glu Ile Ala Pro Thr Gln Val Thr Glu Thr
565 570 575

Asp Glu Cys Arg Leu Asn Gln Asn Ile Cys Gly His Gly Glu Cys Val
580 585 590

Pro Gly Pro Pro Asp Tyr Ser Cys His Cys Asn Pro Gly Tyr Arg Ser
595 600 605

His Pro Gln His Arg Tyr Cys Val Asp Val Asn Glu Cys Glu Ala Glu

610 615 620

Pro Cys Gly Pro Gly Arg Gly Ile Cys Met Asn Thr Gly Gly Ser Tyr
625 630 635 640

Asn Cys His Cys Asn Arg Gly Tyr Arg Leu His Val Gly Ala Gly Gly
645 650 655

Arg Ser Cys Val Asp Leu Asn Glu Cys Ala Lys Pro His Leu Cys Gly
660 665 670

Asp Gly Gly Phe Cys Ile Asn Phe Pro Gly His Tyr Lys Cys Asn Cys
675 680 685

Tyr Pro Gly Tyr Arg Leu Lys Ala Ser Arg Pro Pro Val Cys Glu Asp
690 695 700

Ile Asp Glu Cys Arg Asp Pro Ser Ser Cys Pro Asp Gly Lys Cys Glu
705 710 715 720

Asn Lys Pro Gly Ser Phe Lys Cys Ile Ala Cys Gln Pro Gly Tyr Arg
725 730 735

Ser Gln Gly Gly Gly Ala Cys Arg Asp Val Asn Glu Cys Ala Glu Gly
740 745 750

Ser Pro Cys Ser Pro Gly Trp Cys Glu Asn Leu Pro Gly Ser Phe Arg
755 760 765

Cys Thr Cys Ala Gln Gly Tyr Ala Pro Ala Pro Asp Gly Arg Ser Cys
770 775 780

Leu Asp Val Asp Glu Cys Glu Ala Gly Asp Val Cys Asp Asn Gly Ile
785 790 795 800

Cys Ser Asn Thr Pro Gly Ser Phe Gln Cys Gln Cys Leu Ser Gly Tyr
805 810 815

His Leu Ser Arg Asp Arg Ser His Cys Glu Asp Ile Asp Glu Cys Asp
820 825 830

Phe Pro Ala Ala Cys Ile Gly Gly Asp Cys Ile Asn Thr Asn Gly Ser
835 840 845

Tyr Arg Cys Leu Cys Pro Gln Gly His Arg Leu Val Gly Gly Arg Lys
850 855 860

Cys Gln Asp Ile Asp Glu Cys Ser Gln Asp Pro Ser Leu Cys Leu Pro
865 870 875 880

His Gly Ala Cys Lys Asn Leu Gln Gly Ser Tyr Val Cys Val Cys Asp
885 890 895

Glu Gly Phe Thr Pro Thr Gln Asp Gln His Gly Cys Glu Glu Val Glu
900 905 910

Gln Pro His His Lys Lys Glu Cys Tyr Leu Asn Phe Asp Asp Thr Val
915 920 925

Phe Cys Asp Ser Val Leu Ala Thr Asn Val Thr Gln Gln Glu Cys Cys
930 935 940

Cys Ser Leu Gly Ala Gly Trp Gly Asp His Cys Glu Ile Tyr Pro Cys
945 950 955 960

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Pro Val Tyr Ser Ser Ala Glu Phe His Ser Leu Cys Pro Asp Gly Lys
                965                 970                 975

Gly Tyr Thr Gln Asp Asn Asn Ile Val Asn Tyr Gly Ile Pro Ala His
            980                 985                 990

Arg Asp Ile Asp Glu Cys Met Leu Phe Gly Ser Glu Ile Cys Lys Glu
        995                1000                1005

Gly Lys Cys Val Asn Thr Gln Pro Gly Tyr Glu Cys Tyr Cys Lys Gln
   1010                1015                1020

Gly Phe Tyr Tyr Asp Gly Asn Leu Leu Glu Cys Val Asp Val Asp Glu
1025                1030                1035                1040

Cys Leu Asp Glu Ser Asn Cys Arg Asn Gly Val Cys Glu Asn Thr Arg
                1045                1050                1055

Gly Gly Tyr Arg Cys Ala Cys Thr Pro Pro Ala Glu Tyr Ser Pro Ala
            1060                1065                1070

Gln Arg Gln Cys Leu Ser Pro Glu Glu Met Glu Arg Ala Pro Glu Arg
        1075                1080                1085

Arg Asp Val Cys Trp Ser Gln Arg Gly Glu Asp Gly Met Cys Ala Gly
   1090                1095                1100

Pro Leu Ala Gly Pro Ala Leu Thr Phe Asp Asp Cys Cys Cys Arg Gln
1105                1110                1115                1120

Gly Arg Gly Trp Gly Ala Gln Cys Arg Pro Cys Pro Pro Arg Gly Ala
                1125                1130                1135

Gly Ser His Cys Pro Thr Ser Gln Ser Glu Ser Asn Ser Phe Trp Asp
            1140                1145                1150

Thr Ser Pro Leu Leu Leu Gly Lys Pro Pro Arg Asp Glu Asp Ser Ser
        1155                1160                1165

Glu Glu Asp Ser Asp Glu Cys Arg Cys Val Ser Gly Arg Cys Val Pro
   1170                1175                1180

Arg Pro Gly Gly Ala Val Cys Glu Cys Pro Gly Gly Phe Gln Leu Asp
1185                1190                1195                1200

Ala Ser Arg Ala Arg Cys Val Asp Ile Asp Glu Cys Arg Glu Leu Asn
                1205                1210                1215

Gln Arg Gly Leu Leu Cys Lys Ser Glu Arg Cys Val Asn Thr Ser Gly
            1220                1225                1230

Ser Phe Arg Cys Val Cys Lys Ala Gly Phe Ala Arg Ser Arg Pro His
        1235                1240                1245

Gly Ala Cys Val Pro Gln Arg Arg Arg
   1250                1255
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